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Delivery of the anti-HIV drug azidothymidine (AZT) to T-lymphocytes with neoglycoprotein carriers

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Chapter 1. Introduction

1. Drug targeting systems for antiviral agents: options and limitations

1.1. General considerations

The design of new antiviral agents is often merely based on considerations concerning their desired antiviral activity. Only after the usual ***in vitro*** screening the question arises how the particular compound can be administered ***in vivo*** and delivered to its site of action. It is obvious that the physicochemical features that determine the antiviral activity will intrinsically dictate its fate in the body. Unfavourable pharmacokinetic characteristics can be encountered in this stage of development. For instance, poor oral absorption or bioavailability may occur. In addition, accumulation in tissues leading to overt toxicity can be found. Very rapid elimination from the body and formation of toxic metabolites may imply other drawbacks. Consequently, major problems may be encountered in the clinical phase of testing: severe side effects, large **interindividual** variations in bioavailability as well as an unpractical frequency of administration. Therefore it is preferable to perform early pharmacokinetic screening in the development of antivirals in order to find an optimal combination of **pharmacodynamic** and pharmacokinetic features.

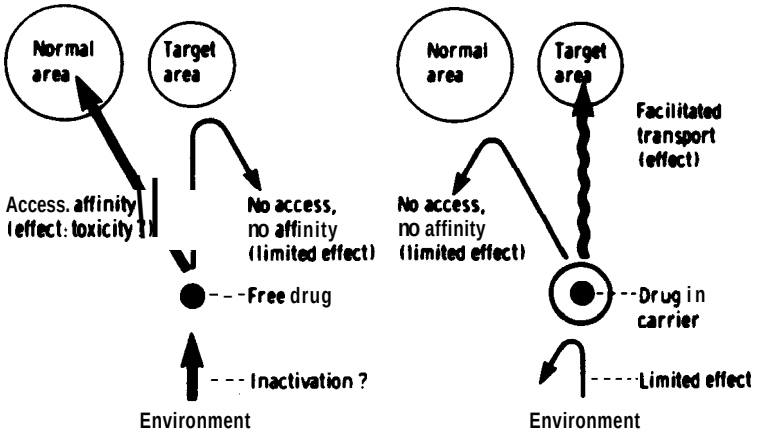


Figure 1. The principle of drug targeting: The disposition of a conjugated drug in the body is determined by the physicochemical properties of the carrier instead of the characteristics of the free *drug.* This leads to higher drug concentrations at the site of action combined with lower concentrations at the non-target tissue where toxicity might occur.

However, even if a satisfactory **bioavailability** of antiviral agents can be attained, only a very small fraction of the bioavailable drug will be directly involved in the antiviral effect. The drug will be mainly distributed to tissues where its presence is not strictly required, or even worse, where it may produce severe side effects. Obviously it would be safer and more economic to deliver the antiviral drug mainly to the cell type where virus replication takes place. This approach is called drug targeting (figs. 1 and 2).

Basically two approaches have been taken to obtain optimal delivery:

1) The **carrier approach:** The **antiviral** drug is covalently coupled to soluble macromolecules or included in particle type of drug carriers **(e.g. nanoparticles** and liposomes). The fate of the drug in the body is now dictated by the chosen carrier. The drug concentration in the target tissue is a resultant of relative rates of cellular uptake of the drug conjugate, liberation of the drug **as** well **as** efflux rate of free drug from the target tissue [1,2] (fig. 3). The advantage of targeting is only significant if the target compartment is different from the compartment where toxicity is occurring and if the active drug is released predominantly at the target site [3].

2) The **prodrug approach:** Chemical derivatives of the drugs are prepared that have more favourable kinetic features and/or are specifically activated at the site of action.

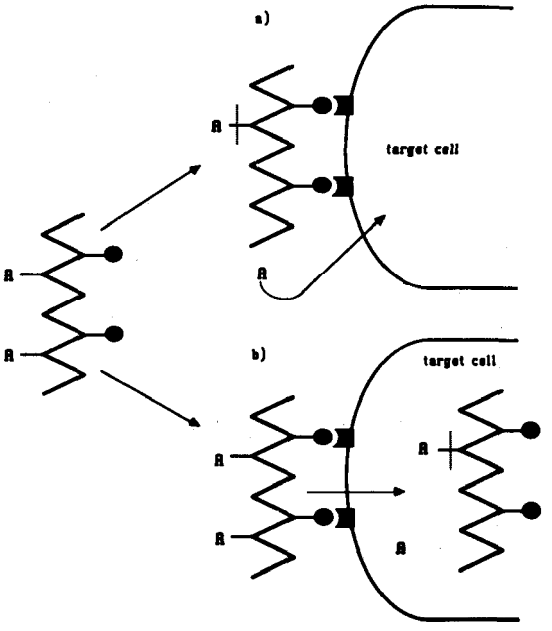


Figure 2. Release patterns of an active agent A after reaching the target tissue: a) release of A outside the target cell, b) release of A inside the target cell. Preferably the release rate should be controllable.

Research aims in drug delivery

The four major strategies in drug targeting are:

a) Slow release targeting. Through association with a suitable carrier, the antiviral drug is delivered into circulating or fixed macrophages. The drug is gradually released from the carrier and diffuses out of the cells, leading to an increased residence time in the body [4].

b) Passive (side-effect avoidance) targeting. Through the association of the drug with a suitable carrier it is prevented from distribution to sites of toxicity.

c) Active targeting. By coupling to a cell specific macromolecular carrier the drug reaches higher therapeutic concentrations at the site of action. Consequently, the dose *can* be reduced and side effects will be minimized.

d) Dual targeting. This implies the use of carrier molecules that have their own intrinsic antiviral effect. With this approach drug conjugates can be prepared with a dual mode of action on the viral replication process: that of the coupled drug in addition to that of the carrier itself. A major advantage is that the virus replication process *can* be attacked at multiple sites potentially leading to synergistic effects and prevention of the development of resistant virus strains.

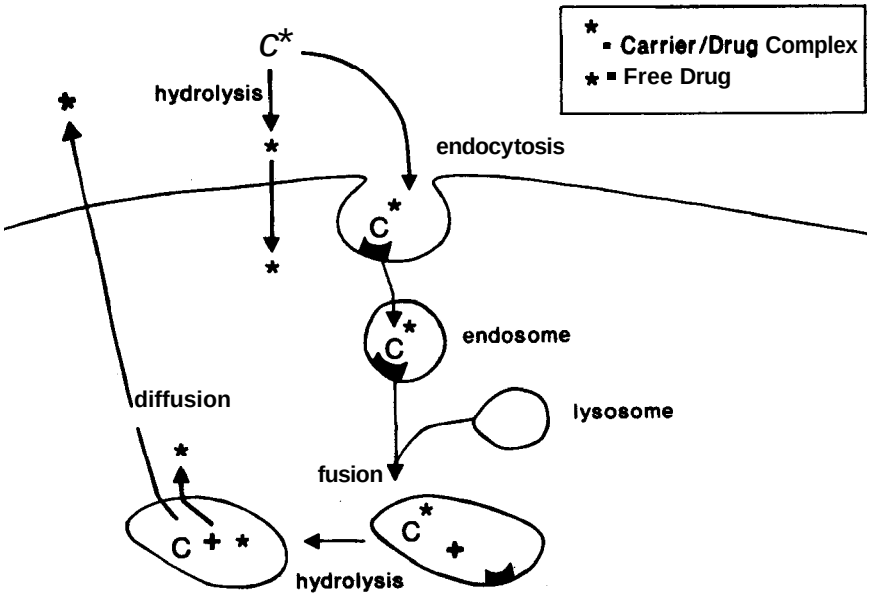


Figure 3. After binding of the carrier to a specific receptor on the target cell, the complex enters the cell via receptor mediated endocytosis and finally ends up in lysosomes. Here the drug is released, diffuses to the cytoplasm and can perform its therapeutic action. The targeting concept assumes cell specific recognition of the carrier, intracellular release and cellular retention of the active *drug*.

It is important to note that especially the last three approaches have provided new perspectives in drug screening. Very potent antiviral compounds with a severe toxicity that were dropped for further testing became serious candidates again as their distribution in the body could be optimized and their therapeutic index largely improved.

Coupling of drugs to macromolecular carrier systems a priori implies that parenteral formulations have to be used. Although parenteral dosing is widely accepted for short term and even for long term clinical use (e.g. insulin and other hormone preparations) it is clear that such drug targeting preparations should have major advantages compared to the parent drug to justify their development. Such advantages could include curing of the disease instead of only slowing down the infection process, therapy of intracellular infections for poorly penetrating drugs or a major reduction of the dosing frequency.

site-specific drug delivery does not prevent the development of steady state plasma concentrations of the parent drug: even if the release rate in the target cells is slow, a part of the drug will tend to enter the general circulation. However, the plasma levels will generally be lower and the local concentration in the target tissue higher. Drug targeting preparations can also be used combined with low doses of the parent drug. This approach could be used if, for instance, steady state levels of the parent drug after administration of a drug delivery form would be too low to provide sufficient therapeutic concentrations in the central nervous system (CNS).

Generally, macromolecular carriers will be unable to deliver drugs into the brain, because of lack of passage over the blood/brain barrier (BBB). However, there is recent evidence that some macromolecules can pass the BBB by transcytotic processes. For transferrin and anti-transferrin receptor antibodies, this process has been clearly established and it was shown that methotrexate can be delivered to the brain in the form of an antibody conjugate [5]. Mannose labeled liposomes were recently claimed to pass the BBB and be delivered to and digested by glial cells [6].

General guidelines in drug delivery research

A number of essential aspects should be mentioned in the design of drug targeting preparations [7-10]:

a) A type of carrier should be chosen that is relatively non-toxic also with regard to its degradation products.

b) The chosen carrier should be able to pass anatomical barriers in the body en route to the target tissues.

c) Drug loading of the carrier should be carefully considered: enough drug molecules should be internalized with the carrier to obtain adequate cellular levels of the drug. However, the coupling of too many drug molecules to the macromolecular carrier may largely perturb its physicochemical features and corrupt the carrier selectivity for the particular receptor system.

d) Cell specific distribution of the drug-targeting preparations as well as the rate of drug release from the carrier should be studied ***in vivo*** both in the normal and the pathological situation. It is of prime importance to check if the chosen drug-targeting concept is also valid in the diseased state. Also, variable sites of viral replication in the various stages of the disease as well as the chronicity of the infection should be taken into account. Promising ***in vitro*** results certainly do not guarantee a similar efficiency ***in vivo.***

e) It is advisable to test drug delivery preparations with regard to possible immunogenicity in an early stage of development, since major problems may occur especially with chronic dosing.

1.2. Reasons for drug delivery of antivirals

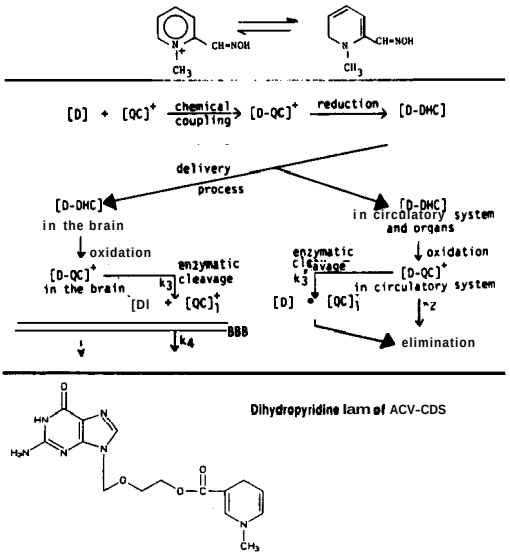
Antiviral drugs are designed with the aim to act on virus-specific processes differing from normal biological events in the host cells. Nevertheless, complete virus-specificity is never obtained and manipulation of the whole body disposition may be necessary to obtain a sufficient therapeutic index.
Features prompting the design of drug targeting formulations for antiviral compounds are:

- Extremely rapid excretion or metabolism, endangering the formation of therapeutic concentrations at practical dosage regimens.
- Severe toxicity in non-target tissue. Examples are bone marrow depression and severe neurotoxicity of vidarabine (ara-A) or formation of very toxic metabolites as recently reported for zidovudine (AZT) [11].
- Poor penetration into the target cells, e.g. poly-anionic agents that are potent reverse transcriptase inhibitors and anti-herpes drugs that poorly penetrate the BBB.
- Inadequate phosphorylation of nucleoside analogues into their active triphosphate forms.
- Poor water solubility leading to limitation in topical administration or precipitation of the drug in the renal tubuli (acyclovir (ACV)).

1.3. Carrier systems

Antiviral prodrugs
This approach consists of the preparation of chemical derivatives leading to prodrugs that have altered pharmacokinetics and/or become activated in virus infected cells. A well known example is acyclovir that is activated by HSV-derived kinases, thereby obtaining an impressive selectivity. Nevertheless, its rather low bioavailability, poor penetration of the blood-brain barrier and poor water solubility are still innate disadvantages. Various esters of ara-A [12] have improved water solubility and in *vivo* efficacy against herpes infections. For instance, the 2', 3'-di-O-acetylester is considered a promising prodrug, combining an optimal balance of water and lipid solubility, deaminase resistance and bioavailability. The tri-acetate derivative of ribavirin has an improved penetration of the BBB and is superior in the treatment of arenavirus-induced haemorrhagic fever in monkeys and of various other viruses [12]. Improved penetration of the BBB was also reported for more lipophilic methyl-substituted dideoxypurines. They may be specifically activated by adenosine-deaminase, an enzyme with increased levels in the CNS during some infective diseases [13]. Various groups have worked on lipid derivatives of nucleoside analogues, such as AZT [14-19], as well as foscarnet [20] and in a number of cases they showed a markedly improved bioavailability, residence time in the body and

antiviral efficacy. An appealing modality is the coupling of various nucleoside analogues to dihydropyridine moieties [21]. Such lipophilic prodrugs (so called chemical delivery systems (CDS)) of ACV, Ara-A and AZT rapidly pass the blood brain barrier after which the prodrug is oxidized to the hydrophilic pyridinium salt. Since the pyridinium group contains a permanently charged quaternary nitrogen and diffusion out of the CNS by passive processes is thereby greatly retarded (fig. 4), the prodrug is trapped in the brain. Subsequent enzymatic ester cleavage of the prodrug is supposed to provide a slow local release of the active antiviral component. For ACV, this leads to 20 fold higher total concentrations as well as a 20 fold increase in residence time in the brain. Although the ACV-CDS complex is also concentrated in other tissues, it disappears more rapidly from these sites compared with the CNS, because these barriers are less absolute. However, the efficacy in the treatment of, for instance, herpes simplex encephalitis and CMV pneumonia, being promising therapeutic objects here, has yet to be studied. A similar prodrug approach has been taken for AZT, showing a tenfold increase in brain exposure and a 30 fold increase in CNS half life, while the potency of the nucleoside prodrug in human lymphocytes was similar to AZT itself [22]. In general, the acute and chronic toxicity of the redox products remain to be studied as well as the relative rates at which the active drug is released intracellularly and extracellularly in target and non-target tissues.



F i e 4. Synthesis and schematic representation of the disposition in the body of dihydropyridine derivatives of nucleoside analogues. After the prodrug is delivered to the brain, the dihydropyridine moiety is oxidized to a permanently positively charged pyridiniumgroup, which is trapped in the brain. The active drug is then slowly released by enzymatic conversion.

Another innovative approach is the chemical coupling of two different antivirally active compounds to yield a prodrug with more favourable properties. Zidovudine and phosphonoformic acid were coupled with the rationale to obtain intracellular release of the substrate- and product analogue that occupy adjacent but not identical binding sites on the RT template. Although the expected synergistic effect was not found, the combination had clearly decreased toxicity, probably related to the gradual intracellular cleavage of the phosphate ester bond.
In the design and testing of antiviral prodrugs, it should be realized that efficacy may largely depend on the time of administration after viral challenge [12]. The replicative cycle of the particular virus as well as the activation stage of the disease should be taken into account in relation to the rates of cellular uptake, activation and degradation of the prodrug preparation.

The microparticle-type of carrier

This category of targeting devices includes liposomes, naturally occurring lipoprotein particles, cellular carriers as well as nanoparticles. An advantage of these types of carriers is that drugs can be simply incorporated in their parent form, without covalent linkage. Either they dissolve in the aqueous phase or become associated with the lipid or polymeric material. To obtain sufficient drug loading, lipophilic derivatives of polar drugs with biodegradable bonds can be synthesized for inclusion in the lipoid phase of liposomes and lipid-particles [23]. Major disadvantages of microparticles are the inability to pass the endothelial lining of the vascular system and, thus, generally poor extravasation [24-28]. Although some investigators claim that slow transcellular (vesicular) transport of liposomes and microspheres is possible in endothelia, their practical application will be largely restricted to intravascular targets. Exceptions to this rule may be well perfused solid tumours and inflamed tissues [7] in which increased extravasation was reported. The mononuclear phagocyte system (MPS), also called the reticulo-endothelial system (RES), efficiently captures exogenous particles either in the parent or in the opsonized form. This process may markedly restrict the contact time of liposomes and nanoparticles with the target cells. If the phagocytosed material is not degraded rapidly enough it may also block MPS function and give rise to chronic toxicity, especially at repeated administration [29]. Various coatings or protectants on the particles, such as negatively charged glycolipids (e.g. phosphatidylinositol, monosialoganglioside or polyethylene glycol (PEG)), have been used to slow down capturing by the MPS and markedly increased the residence time in the circulation [30].

A. *Liposomes*

Liposomes are small vesicles composed of unilamellar or multilamellar arrays of phospholipid bilayers surrounding one or several aqueous compartments (fig. 5).
Charge, lipid composition and size (ranging from 20 to 10,000 nm) of liposomes can be varied and such factors may strongly affect the elimination from the circulation [26,31]. Repeated extrusion through polycarbonate filters with appropriate pore size and microfluidizing techniques can yield very small liposomes (< 100 nm) while most of the entrapped solute is maintained. Sialic acid groups or other polar moieties (for instance PEG) at the liposomal surface may decrease phagocytic uptake and thereby increase circulation time (so called stealth liposomes) [30]. By choosing a proper composition of the liposomes, for instance by including cholesterol and

distearoyl phosphatidyl choline, circulation time is increased and up to 5% of the injected dose can be found in tumours in mice [31a]. Yet the tendency of liposomes to be phagocytosed by the MPS can also be positively exploited in drug delivery [31b]: drugs in microparticles that are accumulated in MPS cells can be slowly released through the rate-limiting biodegradation of the carrier. This results in a 'systemic slow release compartment' that can have major advantages in limiting interdose fluctuation in plasma concentrations of drugs with a small therapeutic index [28]. In addition, accumulation at sites of toxicity can be favourably decreased. Important examples are liposome preparations of the antimycotic agent amphotericine B and of the antineoplastic drug doxorubicin, leading to a 10 fold increase in the therapeutic index (see [24] for references).

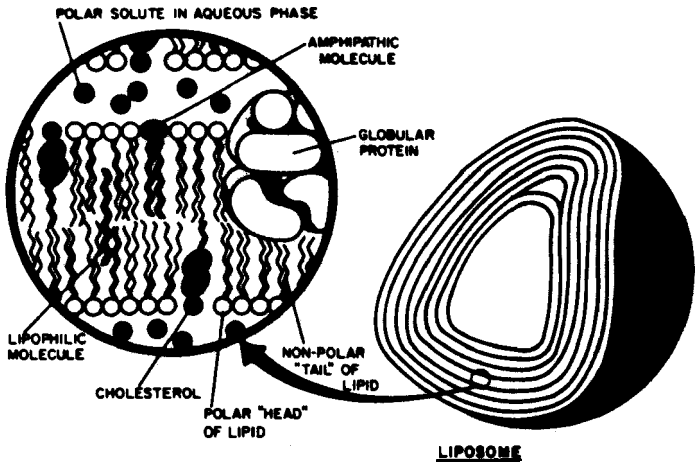


Figure 5. A simplified representation of the structure and composition of liposomes. Potential sites of inclusion of hydrophilic, lipophilic and amphipathic molecules, cholesterol and proteins with multilamellar liposomes are shown (according to Crommelin [28]).

Liposome included drugs may also be more effective in the case of intracellular infections with parasites and bacteria as shown for antileishmaniasis and antimalaria drugs as well as for certain antibodies active against salmonella, mycobacteria and listeria infections [26]. An enhancement of antiviral efficacy has been demonstrated for liposomes containing ribavirin, showing a major therapeutic effect 24 hours after normally lethal inoculation with Rift Valley Fever virus as well as HSV-1 and Influenza A virus [24,26]. Improved antiviral therapy with liposomes has also been reported for murine interferon in a hepatitis model, for zidovudine in a murine immunosuppression model [32], for iodoxuridine in a rabbit HSV model, for phosphonoformate (foscarnet) in the case of HSV-2 infections (see [24] for review) as well as for ganciclovir and foscarnet against CMV fibroblast infections [33]. Foscarnet could be an excellent candidate since after initial delivery into the cells it will only minimally redistribute from the cells due to its polar character [24]. An alterna-

tive antiviral therapy with liposomes is the activation of macrophages with imrnunomodulators such as muramyl peptides [26,34]. Macrophages have been shown to restrict the replication of many different viruses *in vitro* [26]. *In vivo* activation of these cells is not easy because of extremely short half lives and insufficient exposure to the particular lymphokines. Liposomes containing immunomodulators such as N-muramyldipeptide (MDP) and N-muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE) can activate human monocytes to kill HSV-2 infected cells without lysing the uninfected cells. The conversion to a cytolytic state by MTP-PE liposomes occurs at a 800-times lower concentration of muramyldipeptide compared to MDP itself as was clearly demonstrated *in vivo* against various other virus infections. For HIV infections *in vitro* it was shown that liposome included MTP-PE was not only effective immediately after infection, but also reduced virus replication during an established infection. One single treatment was sufficient to obtain maximal effect. Recent clinical studies with liposome-encapsulated MTP-PE demonstrated macrophage activation and antiturnour activity. Tolerance was generally good although dose finding was essential because of a quite narrow therapeutic window [35].

In some of these studies, the control liposomes without the drug also had an effect on virus levels. They reduced the fusogenic ability of HIV-1 and also, to some extent, prevented giant cell formation. Interestingly, this effect was most pronounced for negatively charged liposomes while in contrast positively charged liposomes enhanced virus infectivity [36]. The variable effects of the drug carrier emphasize the importance of liposome composition and also demonstrate that drug carriers should not a priori be seen as inert, unreactive modalities.

Since many human viruses infect and replicate in monocytes-macrophages [26] this cell type is an important target for antiviral compounds. The question arises whether the liposome enclosed drug is resistant to the degradative lysosomal route after phagocytosis and whether it is really delivered intact to the cytoplasm of the cells. In fact, a cytoplasmic distribution is essential for the effects of reverse transcriptase- and protease inhibitors as well as for antisense nucleotides and glycosylation inhibitors [37]. Antibody-bearing liposomes containing antisense oligomers would provide a double specificity: the antibody mediated selection of the particular cell type as well as the selectivity of the mRNA-selected sequence complementary with the liposomally delivered oligomer [38,39]. Yet the antisense component should become available in the cytoplasm. Final delivery of chemically labile components to the aggressive lysosomes could be prevented, at least partially, by the use of pH-sensitive liposomes that are composed in such way that the liposome membranes are destabilized or become fusogenic with the endosomal instead of the lysosomal membranes [24].

Another innovative approach is to mimic the action of enveloped viruses: liposomes could be equipped with virus-derived polypeptides. Such proteins undergo a conformational change at the endosomal pH, resulting in exposure of their hydrophobic residues and fusion with the membranes [24]. Proteins from sendai virus, VSV, SFV, HSV and influenza virus have been reconstituted in liposomes. These 'virosomes' have been mainly tested *in vitro*. It is anticipated, however, that in *vivo* distribution will not be sufficiently cell specific, and that problems of immunogenicity and preparative difficulties will be encountered [40].

A more active type of targeting with liposomes could be achieved by including a

targetdevice at the external surface of the carrier. Examples of such targeting moieties are tissue-specific antibodies [41], glycoproteins [42] and glycolipids. An example of the latter is cetylmannoside for the delivery to human blood monocytes [43]. Lactosylceramide [44] and tris-gal-chol, a cholesterol derivative with terminal clustered galactose groups (fig. 6), were employed to target small liposomes to hepatocytes and larger ones to Kupffer cells [45,46]. Another example is the targeting to peritoneal macrophages with galactosylated liposomes, which were prepared by including a triantennary galactosyl-lysyl-lysine dipeptide [47]. This study demonstrates the importance of optimal geometrics in the recognition of the sugar moiety. An increased tissue specificity can also be obtained by coupling epidermal growth factor [48] or asialoglycoproteins to the liposomes [4,26,27]. An alternative is reductive lactosylation of the apolipoprotein portion of LDL and HDL particles [49].

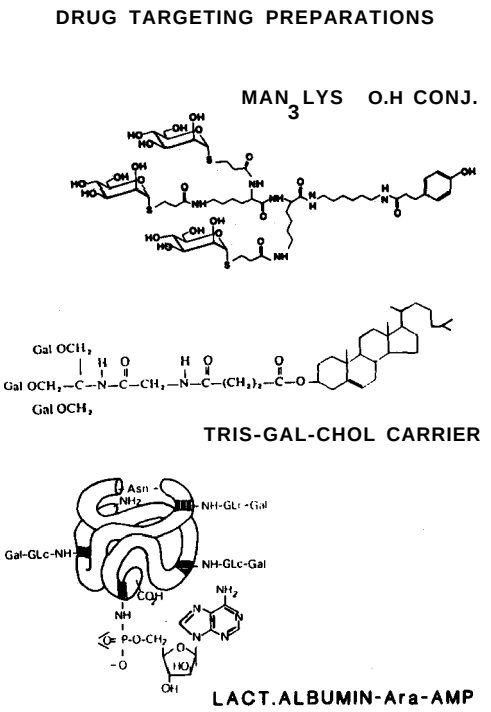


Figure 6. Artificial drug carriers based on sugar recognition. **Polylysine** can be **derivatized with** clustered mannose groups and **provides** a carrier for **Bolton-Hunter** reagent (BH) to macrophages (top). **Cholesterol can** be **connected** via a **tris-spacer** to three clustered galactose groups and can be **included** in LDL **and** HDL particles, targeting them to sinusoidal **cells and hepatocytes** through recognition by the galactose particle receptor and the **galactose/N-acetyl** galactosamine receptor (hepatic lectin), **respectively** (mid). **Ara-**AMP is **covalently** linked **via an acid labile phosphoamide bond** to randomly **lactosaminated** albumin and is thereby targeted to **hepatocytes (bottom).**

An important tool in site-specific delivery of liposomes may be the use of immunoliposomes: liposomes to which antibodies or fragments of antibodies are attached using various hetero-bifunctional cross-linking reagents [4,25-27,50,51]. However, *in vivo* studies demonstrated that immunoliposomes still exhibited major uptake by cells of the MPS. This process could be reduced in favour of the target cells by prior administration of lysosomal inhibitory agents or large amounts of 'dummy liposomes'. The latter aspect implies that whole body distribution of liposomes is in principle dose dependent [50,52a]. Recently, Maruyama et al. achieved optimal binding and retention by the mouse pulmonary endothelial cells with immunoliposomes if the lipid composition included ganglioside (GM1) to reduce the uptake of the immunoliposomes by the MPS [52b].

A recent development is the design of target-sensitive immunoliposomes [53,54]. They are composed of palmitoyl antibody stabilized phosphatidyl ethanolamine bilayers that are destabilized upon binding to an antigen on the target cell leading to a local unloading into the cytoplasm of the target cells. *In vitro* this resulted in a 1000 fold increase in uptake of ACV compared with the free drug. *In vivo* testing of the target-sensitive liposomes should give more definite clues as to the applicability.

Finally, a number of additional potential drawbacks for the practical use of liposome preparations should be mentioned. First of all it should be realized that intracellular delivery of some nucleoside analogues with liposomes may not be effective since the drugs are rapidly degraded in lysosomes [24,40]. Other obstacles for liposome mediated delivery are opsonisation by circulating antibodies (or by antigens in the case of immunoliposomes), leading to clearance mediated by Fc-receptors. In addition, heterogeneity of the target cells may corrupt the immunoliposome concept [52]. Subcutaneous, intraperitoneal, pulmonary or topical application of liposomes leading to transport to lymphatics and lymph nodes may circumvent some of the above mentioned problems [26,40].

A general warning should be put forward with regard to the design of site-directed drug targeting preparations: if endocytosis is required for cellular delivery, virus infected cells may be much less active in endocytosis, for instance due to depletion of energy rich metabolites or decreased expression of cell surface receptors. The delivery concept may thus be severely invalidated in case of particular viral diseases [54].

B. LDL and HDL *particles*

Endogenous lipid particles such as LDL (25 nm diameter) and HDL (10 nm diameter), containing a lipid and an apoprotein part, can be viewed upon as a sort of 'natural targeted liposome': the lipoid core can be used to incorporate lipophilic drugs or lipophilic prodrugs. The apolipoprotein part of the particles can be glycosylated. Consequently, cell specific recognition by receptors other than the physiological LDL and HDL receptors can be attained [45,46,55]. Alternatively, the lipid component of these particles can be equipped with glycolipids that expose their sugar groups and are instrumental in delivery to sugar recognizing lectins on various cells types in the body (table I).

The dimensions of LDL and HDL particles predict that they will not easily pass normal endothelial barriers apart from the fenestrated linings in liver and spleen and possibly endothelia in tumours.

C. Microspheres and nanoparticles

This *type* of carrier often consists of biocompatible polymers. Both soluble and particulate carriers have been produced. The soluble carriers have the advantage that large amounts of drug can be incorporated. Up to 80% (w/w) of these soluble carriers can be used for drug loading [50,56,57]. Dextrans, ficoll, sepharose, heteropolysaccharides, poly-L-lysine and N-hydroxy propyl methacrylamide (HPMA) copolymers are well known examples. They have been applied for instance for delivery of amantidine and interferons [12]. The polymeric backbone of the carrier can also be derivatized with specific sugars aimed at the various cell surface lectins in the body [58,59].

Nanoparticles (0.2-0.5 pm) have been prepared from polyalkyl-cyanoacrylates [60], and microspheres from denatured albumin (diameter of 30-200 pm). They have a lower payload than the soluble polymers. Drug loading may occur by simple a b sorption or real incorporation [61]. An advantage can be the non-covalent binding of the drug. Nanoparticles can also be equipped with antibodies or Fab' fragments [60]. Yet, after in vim administration, there is rapid clearance by cells of the MPS. Opsonisation of nanoparticles with immunoglobulins, fibronectin or complement factors in the intact organism may be at least partially responsible for the in *vivo* routing to the cells of the MPS [52]. Therefore, especially intracellular infections in Kupffer cells and other macrophages may be useful targets. Coating of these particles with PEGs does not divert such polymeric microspheres from the MPS cells of liver and spleen [50,52]. Phagocytosis or adsorptive endocytosis of nanoparticles into macrophages will certainly activate the immune system and may largely increase the immunogenic response toward the drug and/or its carrier, representing a potential drawback with chronic dosing of such preparations. Albumin microspheres with a size exceeding 100 μm may become entrapped within capillary networks that they encounter after i.v. administration [62,63].

D. Cellular carriers

Cellular carriers have been extensively studied [8,62] but relatively few practical applications are available to date. Blood derived cells such as (modified) red blood cells (RBCs), leucocytes, lymphocytes and fibroblasts have been employed as storage sites for drugs or cellular transplants [8,40]. Cellular carriers may have the advantage of the natural biocompatibility. However, they will encounter the endothelial barriers and are unable to fuse with other cells. Nicolau et al. [64] designed long-lived CD4-bearing RBCs by electro-insertion of CD4 molecules. These cells were claimed to be capable of clearing free HIV and gp 120 and allow binding of HIV-infected cells. The latter may lead to cellular aggregates that should be easily removed by phagocytosis, but in principle may also lead to severe toxicity.

Soluble type of carriers

A. *Receptor based drug targeting in viw*

The success of drug targeting with macromolecular carriers is intimately depending on the selectivity in the distribution of the cellular receptor targets in the body. Other crucial factors are the anatomical and/or pathological barriers that have to be passed en route to these recognition sites [7,10,58,65]. Table I lists a number of receptors for macromolecules that have been identified and that are more or less specific for the cell type indicated. Many of such receptors are lectins that recognize oligosaccharide

chains in a specific geometric arrangement, with a pronounced role for the type of the terminal sugar (see fig. 7). In addition, such receptors may differentiate on the basis of overall charge and the charge density of macromolecules [66]. Often these receptors also provide mechanisms for internalization followed by intracellular transport to degradative compartments. At these sites, the coupled drug molecules should be liberated through biodegradation of the carrier. In some cases, however, only external binding is offered. A local release of drug from the carrier at the microclimate of the cell membrane should then provide a sufficient driving force for uptake into the target cell [67]. Both receptor affinity and density as well as the presence of competing endogenous ligands determine the extent of carrier-receptor occupation and thereby the extraction of the carrierdrug complex by the target tissue.

Table 1. Whole body distribution of various receptors specific for their preferred **ligands**

Cell	Receptor
Hepatocytes	**galactose-t(n)GP**[1] (high density), HDL, LDL, EGF, **IgA,** transferrin
Kupffer cells	galactose particles, **mannose-t(n)GP,** LDL, AMPC, polymeric negatively charged proteins, complement factors
Fibroblasts	mannose-6-phosphate-t(n)GP, transferrin, LDL, EGF, transcobalamin **II,** AMPC
Mammary acinar cells	growth factor
Endothelia	
- **Blood/brain**	**transferrin,** insulin
- **Diaphragma,** lung, heart	albumin
- Liver	monomeric negatively charged proteins, **man/glcNAc-t(n)GP,** Fc receplor
Enterocytes	maternal **IgG,** dimeric IgG, **transcobalamin II.**
Monocytes	mannose-6-phosphate-t(n)GP
Macrophages	**man/glcNAc-t(n)GP, ß-galactose-t(n)GP, mannose-6-phosphate-t(n)GP,** AMPC
CD4+ T-cells	**galactose-t(n)GP** (low density), **CD4, interleukin, transferrin**
Renal tubular cells	low molecular weight proteins **(cationic)**

[1] Abbreviations used: **t(n)GP** = terminated **(neo)-glycoproteins,** IgA = Immunoglobulin A, EGF = epidermal growth factor, AMPC = α_2**-macroglobulin protease** complex.

At least five mammalian receptor systems with a predominant hepatic distribution are available for the clearance of glycoproteins (table I). Most of these processes have been studied in detail with regard to cellular kinetics. Depending on the rate and site of ligand-receptor dissociation within the cells, intracellular trafficking can occur to endosomes and lysosomes but also (albeit to a lesser extent) to other cell organelles such as Golgi-apparatus, mitochondria and the cell nucleus. The relative rates of acidification of the intracellular vesicular compartments may largely determine intracellular sorting and routing [68]. It is important to note that continuous exposition of certain receptors to their macromolecular ligands can lead to rapid downregulation of cell surface receptors since receptor recycling within the cells is not complete [69]. This downregulation should be taken into account in predicting pharmacokinetics of carriers. For instance, when the particular target receptors are present on more than one cell type in the body, and downregulation in these cells occurs at different rates, tissue specificity for drug-carrier complexes in the body may change in time during chronic dosing. Similar types of sugar-recognizing receptors for glycoproteins are also present (albeit to a smaller extent) on blood cell types (see chapter 1, part 3) [59,70].

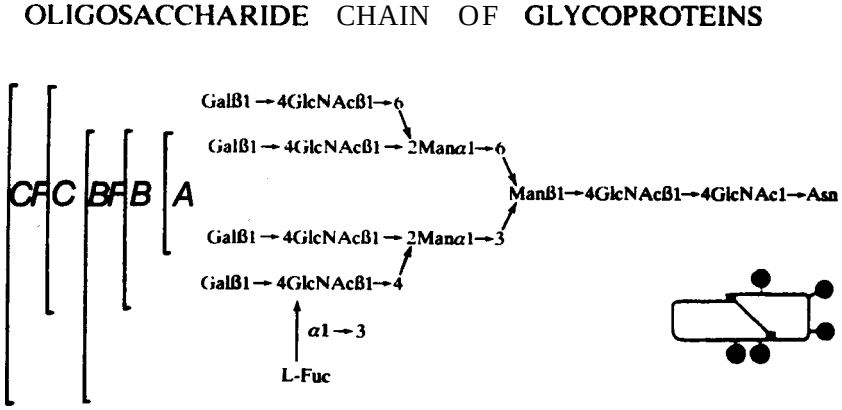


Fig 7. Characteristic structure of the oligosaccharide side chains of naturally occurring glycoproteins, such as al-acid glycoprotein (orosomucoid) and fetuin. Normally the major part of the galactose groups is connected to N-acetyl-neuraminic acid (sialic acid). Microheterogeneity in the antennary structure as well as in sialic acid content exists under pathological conditions. A, B and C denote bi-, tri- and tetraantennary structures, respectively. In the CF and BF forms L-fucose is present at the indicated site. The inset shows localization of five of these chains at the orosomucoid polypeptide moiety, having two subunits connected with disulfide bridges.

Depending on the stage of the disease, the expression of normal surface receptors can largely vary. For instance the galactosyl-receptor on hepatocytes is poorly expressed in primary hepatic cancer cells [71]. Receptor density or the efficiency of receptor mediated endocytosis can also be affected by virus infection of the particular cell type [10].

It is interesting to note that many viruses have envelope glycoproteins that use well defined cell surface receptors for virus binding and entry. In fact, viruses can be regarded as delivery systems for genetic material and could be employed as modalities for cell-specific gene- and drug targeting [50,72]. Polypeptides or proteoglycan-carriers could be designed that would mimic such virus-specific recognition and could be exploited to target antiviral drugs to those cells in which virus replication would take place [24]. Along these lines, carriers could be employed that specifically react with viral envelope proteins exposed at the surface of the infected cells.

B. Properties of soluble macromolecular carriers

The major difference between the soluble type of carriers and the particle type is their size. The size of the particles ranges from 100 μm down to 0.1 pm, whereas the soluble carriers are much smaller, with a molecular weight of less than 200 kD and a 1-2 nm size. The small size may provide some advantages but also certain disadvantages.

One of the major advantages of soluble carriers over the particle type is that the former can easily leave the systemic circulation. Consequently they can be used to target drugs to cells that are not in direct contact with the blood. Another advantage is that they are not necessarily removed by the MPS.

Soluble carriers usually have a relatively low loading capacity of less than 50 drug molecules per carrier, whereas liposomes and LDL particles may carry up to 500 molecules per particle. However, since liposomes have a larger size, this difference in loading capacity is much less when comparing an equal volume of both carrier systems. The drugs have to be covalently linked to the soluble carriers and to assure release of the active drug at the target site, biodegradable or acid-labile spacers need to be used [67,73]. Acid labile spacers between drug and carrier for release in intracellular acidic compartments and also specific peptide spacers that can only be cleaved by enzymes present in the target cells were recently developed in our laboratory [74].

C. (Monoclonal) Antibodies

The use of (monoclonal) antibodies ((Mo)Abs) to target drugs to specific cell types is a promising approach in view of the large extent of tissue specificity that is obtained. Drugs are coupled to an antibody (fig. 8), thereby creating a hybrid molecule with the specificity of the immunological ligand retaining the therapeutic activity of the drug [75].

Serious disadvantages that can be anticipated using immunoconjugates include:

a) specific toxicity of the antibodies due to their cross reactivity with non-target cells [75],
b) limited access of the Ab preparations to the target cell type [76],
c) cell heterogeneity with respect to the determinant to which the antibody is directed [76,77],

d) **immunogenicity** of the Ab and especially that of the **Ab-drug** conjugate,
e) **'opsonisation'** of injected **Abs** and complex formation with circulating antigens.

The importance of the latter point was clearly demonstrated by the observation that tumour-specific antibodies have largely different **pharmacokinetics** in **tumour-bearing** individuals compared to normals because of a rapid clearance of the immune complexes by the liver **[78]. Immunotoxins** may also be partly inactivated and cleared via the α_2-**macroglobulin** system and subsequently accumulate in non-target tissue such as liver and monocytes **[79].**

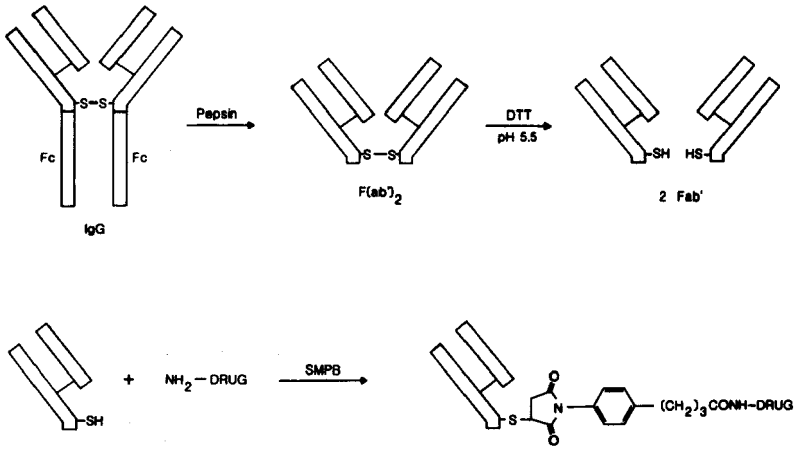


Figure 8. Covalent coupling of drugs to Fab' fragments. $F(ab')_2$ dimers are prepared by pepsin digestion of the IgG molecules. Fab' monomers are generated from these dimers by reduction with dithiotreitol (DTT) at low pH. The drug is covalently attached to the Fab' fragments using SMPB (according to Crommelin [28]).

The potential problems with the Ab carriers can be tackled in various ways. Purification of the antibody, using affinity techniques, can be used to extract the cross reacting fraction **[80].** The access of Abs to the cells can be improved by employing immunological active fragments (Fab' and $F(ab')_2$) instead of the complete **IgG** protein [80]. Transcapillary and cell membrane passage of these fragments is much better than that of the intact immune products **[77,80].** In addition, such fragments display a diminished immunogenicity compared to the entire protein **[80].** Yet, the most attractive targets for **immunoconjugates** remain **(intra)vascular** cell types such as lymphocytes, monocytes, macrophages and capillary endothelial cells. **Pretolerisation** of patients by immunosuppressive monomethoxypolyethyleneglycol **(mPEG)** conjugates prior to the treatment with the immunoconjugate itself may reduce the immunogenic reactions **[81].**

Immunotoxins

Toxins are molecules that inactivate vital cytosolic components of the protein synthesis machinery in a catalytic manner. A major requirement for a therapeutic effect of toxins, therefore, is to reach the cytosol of the target cell [77,82]. Immunotoxins (ITs) are conjugates of antibodies and toxins in which the cell binding moiety of the toxin is replaced by the binding specificity of an Ab. Although ITs were originally developed for the treatment of various tumours, the concept is also applicable in antiviral therapy [82]. Ricin toxin is one of the most commonly applied and studied compounds. It consists of two chains, the A- and B-chain. The A chain is responsible for the cellular activity, whereas the B-chain is in fact a galactose specific lectin responsible for the binding to virtually all eukaryotic cells [75]. Replacing the B-chain with a monoclonal antibody therefore provides a greatly improved cell specificity. Mice with ongoing graft versus host disease were treated with MoAbs coupled to Ricin A-chain, resulting in a significantly increased survival time. However, protection lasted only as long as the IT-A was administered [83]. Studies in rats and mice showed that ITs of Abs and Ricin A-chain are rapidly taken up by various liver cell types, due to the presence of mannose-rich oligosaccharides in the A chain. Deglycosylation of the A-chain resulted in an IT-dgA with a much longer half life in *vivo* [84].

Investigations by Hwang [76], using the more potent Abrin toxin A-chain, showed that different cell types expressing the same antigen are not necessarily equally susceptible to the cytotoxicity of a conjugate consisting of the same MoAb.

For treatment of HIV infections, highly specific ITs can be developed directed to specific viral antigens on the cell surface of infected cells or aimed at the highly conserved CD4 molecule on CD4 positive T-lymphocytes or on several other cell types [85-87]. However, the therapeutic aim is to specifically destroy infected cells. Although MoAbs directed against HIV-1 envelope gp120 have a high selectivity for infected cells, this approach may be corrupted due to the heterogeneity of gp120 among the various HIV isolates. Furthermore, circulating free gp120 may bind to healthy CD4 positive cells and convert them into target cells for the MoAbs [88]. Immunotoxins of Ricin A-chain or *Pseudomonas* Exotoxin and MoAbs directed against gp120 inhibited production of infectious virus in HIV infected cells, and did not affect non-infected cells *in vitro* [89,90]. However, the authors recognized the problem of gp120 heterogeneity and proposed to use panels of broadly reactive ITs *in vivo* to avoid variant virus selection. In contrast to gp120, HIV fusion glycoprotein gp41 is only expressed on infected cells. Its structure is highly conserved, resulting in highly specific anti-gp41 MoAbs [88].

Barbieri [91] used an IT composed of anti-CD4 MoAb and Saporin, a ribosome inactivating protein. This Saporin-IT was effective and selective in killing CD4 positive cells. In contrast, anti-CD4- and anti-CD8- Ricin A-chain ITs were found to be ineffective against CD4 and CD8 positive cells, probably due to a poor internalization. It was concluded that internalization of ITs may occur through the toxin moiety rather than through the antibody part and that Saporin is taken up by cells through a mechanism different from that operating in the case of Ricin A-chain. Immunoconjugates, recognizing the CD4 binding region or a variable region of gp120 and containing pokeweed antiviral protein, were able to kill H9 cells infected with various HIV-strains *in vitro* without affecting non-infected cells. They retained their ability to neutralize HIV virions and prevent syncytium formation [86].

Anti-gp41-dgA ITs were shown to have a very selective and potent toxicity towards HIV-infected H9 and U937 cells [88]. The cause of this remarkable cytotoxic potency may be the proximity of the target epitope relative to the plasma membrane. This may facilitate internalization of dgA and routing of dgA to intracellular compartments that are instrumental in translocation of the A-chain into the cytosol. Experiments performed in the presence of an excess of unconjugated anti-gp41 Ab only partially inhibited IT-induced cytotoxicity. This may suggest that the presence of anti-gp41 Abs in the serum of patients may not be an obstacle for therapy with this type of immunoconjugates [88].

Antibody-enzyme conjugates

A novel strategy for the delivery of cytotoxic or antiviral agents to specific cell types is the prodrug activation by antibody-enzyme conjugates. The enzymes to be targeted are chosen for their ability to convert relatively non-toxic drug precursors into their active form. Such antibody coupled enzymes can then be specifically delivered to the cell type that expresses the antigenic determinant [92]. The formation of active drugs in the close proximity of the target cells could lead to higher cellular and lower systemic concentrations of the active drug. In fact, the need for Ab internalization, one of the major problems in immunoconjugate application, is eliminated in this concept. Enzymes that can be used are for instance alkaline phosphatase (for the conversion of phosphated prodrugs) and carboxypeptidase (an enzyme converting some inactive carboxy compounds into their active carboxylic counterparts). Although this strategy has only be described in cancer therapy so far [93,94], it may also be applicable to the therapy of viral infections. A potential drawback forms the presence of related (endogenous) enzymes in the general circulation of the treated individual that may lead to premature activation of the particular product.

Bifunctional antibodies

An important development is the targeting of bifunctional antibodies. For instance, linkage of an antitumour antibody with an antilymphocyte antibody, produces a bifunctional protein that can redirect T-lymphocytes to lyse tumour cells [95]. A similar procedure was proposed for toxic T-lymphocytes killing HIV-infected cells [96].

D. *rCD4-toxin conjugates*

In the search for more effective anti-HIV agents, several groups reported on the use of recombinant CD4 (rCD4) as a carrier for toxins. In principle, rCD4 can exhibit the same degree of cell specificity and affinity as antibodies do. rCD4 was chosen because of its high affinity for the HIV envelope gp120. Although gp120 shows a distinct variability among different HIV strains, its CD4 binding site is highly conserved. Therefore, rCD4 may exhibit the same high affinity to gp120 as cellular CD4 and would potentially target coupled antiviral drugs to HIV infected cells.

The loss of CD4 positive cells in HIV patients is partly due to the interaction of gp120 in the membrane of infected cells with CD4 molecules of non-infected cells. This leads to cell membrane fusion and syncytium formation and implies that rCD4 carriers will have an additional intrinsic therapeutic effect. A pronounced anti-HIV activity of a conjugate of rCD4 with deglycosylated ricin A chain was found in a HIV infected human T-cell line. Non-infected cells expressing MHC II antigens (the natural ligand for CD4) were not affected [97]. A recombinant protein, containing the HIV-binding portion of the human CD4 molecule, was linked to active regions of

Pseudomonas Exotoxin A (PE-A) and displayed selective toxicity towards cells expressing the gp120 [98]. A combination of this CD4-PE-A and reverse transcriptase inhibitors resulted in highly synergistic effects and led to a complete elimination of infectious HIV-1 from human T-cell lines ***in vitro*** [99]. In spite of these promising in ***vitro*** effects, further ***in vivo*** testing is awaited. Major problems may be encountered in the immunogenicity of the rCD4 products as well as in harmful effects caused by interactions with elements of the immune system.

E. Soluble polymers

Soluble synthetic polymers have been widely employed to design versatile drug carrier preparations. Polymer chemistry allows the introduction of targeting devices into the backbone of the polymeric carrier molecule essential for cell specific drug delivery [57]. A larger number of potential target sites in the body can be reached compared with the microparticulate types [100].

Polymeric carriers should not adhere non-specifically to cells and therefore excessive charge or hydrophobicity of the polymers has to be avoided. In addition, the molecular weight should be large enough to avoid glomerular filtration in the kidneys and small enough to reach the target cells [100].

N-(2-hydroxypropyl)methacrylamide (HPMA) polymers have been extensively studied as drug carriers. However, they have the disadvantage that the main polymer chain is not biodegradable. Crosslinking of relatively small HPMA polymers with oligopeptides, however, leads to intracellular degradation of the cross links and excretion of the polymer breakdown products [101]. The introduction of oligopeptide structures also provides suitable attachment moieties for drugs [102]. The immunogenicity of conjugates based on copolymers of HPMA is claimed to be low [102].

One important application of soluble peptide or polymeric carriers would be the delivery of antiviral antisense oligo-deoxynucleotides. Multiplication of Rous Sarcoma Virus, Vesicular Stomatitis Virus, Herpes Simplex Virus, Influenza Virus and HIV-1 can be inhibited using synthetic oligodeoxyribonucleotides that specifically hybridize to complementary DNA or RNA sequences. However, the antisense concept has two implicit drawbacks. Oligomers are quite sensitive to circulating nucleases and exhibit poor penetration into the target cells [103,104]. One way to improve the cellular uptake of the polyanionic oligomers, is the coupling to hydrophobic moieties. For instance, oligo-deoxynucleotides containing methylphosphonate are rapidly taken up in CHRCS cells [105]. Furthermore, linking an undecyl group to the 5' terminal phosphate of antisense oligomers, effectively inhibited influenza virus reproduction, while the non-modified antisense oligomer had no effect [106]. Conjugation to cell-specific macromolecules could also provide a potential solution to this problem. Degols et al. [107] reported a 90 to 99% inhibition of VSV multiplication in L929 cells with an antisense oligomer-***poly-L-lysine*** conjugate at concentrations far below those needed for the uncoupled oligomers. Stevenson et al. showed that at concentrations as low as 200 nM, poly-L-lysine modified oligomers prevented MT-4 cells from HIV-1 induced cytopathic effects [108]. Although this delivery system is useful for increasing the cellular availability [109] and the metabolic stability of the oligomers, poly-L-lysine is rather toxic at high concentrations and also ineffective in some cell lines [107]. Therefore other types of soluble macromolecular carriers are worthwhile to consider.

Transferrin-poly-L-lysine and transfertin-protarnine were complexed with **plasmid** DNA and shown to be efficiently bound to and endocytosed by haematopoietic cells, leading to the expression of the transferred genes **[110].** Expression of **transferrin** receptors on proliferating cells could render these cells susceptible to **intracellular** delivery of antivirally active drugs or antisense oligomers.

F. Glycoproteins

Many enzymes, acute-phase proteins and most plasma proteins (apart from albumin and **lysozyme)** are glycoproteins. The principal sugars forming the oligosaccharide chains are **mannose,** N-acetylglucosamine **(glucNAc),** galactose and sialic acid (N-**acetyl-neuraminic** acid) **[111,112].** Figure 7 gives a schematic representation of the oligosaccharide side chains of orosomucoid, a glycoprotein used in many experimental studies **[8,113].**

The classic publication of **Ashwell** and **Morell** in 1974 **[114],** describing the rapid plasma clearance of desialylated **ceruloplasmin** in rabbits disclosed a large field of research in studying the disposition of circulating glycoproteins **[112].**

The terminal sialic acid residues on the carbohydrate moieties of glycoproteins are regarded essential for the normal survival of these compounds in the circulation. A glycoprotein which is desialylated exposes terminal galactose groups and is cleared much more rapidly from the circulation than the corresponding native glycoprotein. The galactose groups are recognized by a specific receptor on the plasma membrane of hepatocytes called the asialoglycoprotein receptor (ASGPR). It has been studied with a large number of desialylated plasma glycoproteins like orosomucoid, fetuin, lactofemn, a,-macroglobulin and haptoglobulin **[1**11,112,1151.

After this first discovery of a galactose recognizing (lectin-like) receptor on hepatocytes, several authors have reported receptors on other cell types with an affinity for other sugars (table I). For example, a **mannose/glucNAc** receptor on alveolar macrophages and sinusoidal liver cells **[1**16-1**18]** and a galactose terminal **biantennary** oligosaccharide recognizing receptor on T-lymphocytes **[119].**

Glycoproteins represent excellent objects for drug targeting **[8,120].** Preparation is relatively cheap compared with liposomes or antibody-drug conjugates. Moreover, the structure can be easily modified with regard to the protein backbone as well as the functional sugar groups. So far naturally occurring plasma proteins such as **orosomu**-coid (a,-acid glycoprotein) and fetuin were often used. They display a relatively high affinity for the asialoglycoprotein receptor probably due to the clustered arrangement of the antennary oligosaccharide side chains **[121].** Table II summarizes the use of various carrier molecules with different antiviral drugs in several viral diseases.

A major problem of naturally occurring plasma proteins is the presence of the **non**-terminal sugars in the oligosaccharide chain that invite interactions with other receptors in the body. Enzymatic cleavage of sugars from the chain in order to expose the required terminal sugar is often incomplete. With this procedure, glycoproteins with a mixture of terminal sugars are produced and consequently a loss of cell specificity should be anticipated.

Some artificial carrier systems mimic this geometric organization of sugar groups. Plasma proteins such as albumin and **apolipoprotein** B can be randomly derivatized with various kinds of sugar molecules. In this manner neoglycoproteins are produced with a well defined amount and type of exposed sugar. One method for sugar coupling to **peptides** is the reductive amination using boronhydride and a disaccharide

e.g. lactose [129]. In this reaction the aldehyde moiety of glucose reacts with ϵ-NH_2 groups of **lysine,** resulting in a terminal galactose moiety. The nitrogen-atom in the protein-sugar linkage can still be protonated and consequently no positive charge is lost in this coupling reaction. Nevertheless, conformational changes and loss of flexibility in the protein molecule due to sugar derivatization can occur.

Table II. Summary of reported drug targeting approaches in antiviral research.

Drug	Carrier	Virus	Cell type	Receptor	Exp. Meth.	Ref.
ara-AMP	lactose-HSA	**Ectromelia**	hepatocytes	galactose	in mouse	**[122]**
ara-AMP	galactose **poly-lysine**	**Ectromelia**	hepatocytes	galactose	in mouse	**[123]**
ara-AMP	lactose-HSA	HBV	**hepatocytes**	**galactose**	in man woodchuck	**[124]** **[125]**
trifluoro-thymidine	**asialo-fetuin**	**Ectromelia**	**hepatocytes**	galactose	in mouse	**[123]**
PMEA	**mannose-poly-lysine**	HSV-1	**macrophages**	**mannose**	in vitro	**[59]**
Ricin A	**rCD4**	**HIV**	**CD4+** lympho-cytes	**gp120**	in vitro	**[97]**
Pseudom. Exotox.A	**rCD4**	**HIV**	**CD4+** lympho-cytes	gp120	in vitro	**[99]** **[98]**
Ricin A Pseudom. **Exotox.A**	anti-**gp120** MoAb	HIV	**CD4+** lympho-cytes	**gp120**	in vitro	[90] **[89]**
Saporin	anti-**CD4** MoAb	**HIV**	**CD4+** lympho-cytes	**CD4**	in vitro	**[91]**
Ricin A	anti-**gp41** MoAb	HIV	H9 cell line **U937** cell line	gp41	in vitro	**[88]**
Oligodeoxy-ribonucl.	**poly-L-**lysine	VSV	**L929** cell line	none	**in vitro**	**[107]**
Ribavarin	**liposome**	RVF HSV-1 **Influenza**	?	none	in **vivo**	[26]
Interferon	**liposome**	HBV	hepatocytes	none	in vivo	**[24]**
Foscarnet	**liposome**	HSV-2	?	**none**	in vivo	**[33]**
		CMV	fibroblasts	nonc	in vivo	**[33]**
MTP-PE	**liposome**	HSV-2	?	**none**	in vivo	**[35]**
Ricin	anti-CD4 MoAb		**CD4+**	**CD4**	in **vitro**	**[87]**
	anti-CD8 MoAb		**CD8+**	**CD8**	in vitro	**[87]**
Pokeweed antiviral protein	**anti-gp120** MoAb	HIV	**H9 cell line**	**gp120**	in vitro	**[86]**
	anti-CD4/ CD5/CD7 MoAb	HIV	**CD4+/CD5+ CD7+**	**CD4,CD5 CD7**	in vitro	**[85]**
DAB_{486}	1G2		**IL-2R+**	**IL-2R**	in vitro	**[126]** **[127]** **[128]**

Other methods to connect sugars include the thioglycoside method [121] and coupling via thiophosgene activation of para-amino-phenyl sugars [130]. The latter method yields relatively negatively charged proteins since the nitrogen-atom in the linking moiety cannot be protonated in contrast to the lysine ϵ-NH_2 groups in the parent protein. Competition studies and immunohistochemistry indicated that these poly-anionic compounds are no longer solely recognized on the basis of the connected sugar groups, but are endocytosed via other receptors on sinusoidal liver cells, for example a receptor on Kupffer cells that recognizes both the particular terminal sugars and the net negative charge [66]. In addition, a scavenger receptor is described mediating the removal of polyanionic proteins, which is mainly present on endothelial liver cells [131]. Also, subsequent covalent coupling of drugs to the remaining ϵ-NH_2 groups may further increase the negative charge. Drugs with acidic functional groups will amplify this problem. For instance, coupling of fluoresceine-isothiocyanate (FITC) will add two negative charges per coupled molecule. The coupling of three FITC molecules to one albumin molecule converts albumin to a proper ligand for the above mentioned scavenger receptor [132].

Chemical moieties used to link drugs to carrier proteins should be stable in the bloodstream to prevent premature degradation. Such linkages should be labile in acidic compartments within the target cells. For instance, antiviral nucleoside analogues in their mono- or diphosphate forms can be linked to lysine ϵ-NH_2 and histidine nitrogen via a pH-sensitive phosphoamide group [133] (fig. 6).

Release of nucleosides in the polar monophosphate form may be crucial for keeping the drug inside the cells. Non-phosphorylated nucleosides such as trifluorothymidine were shown to rapidly leak out of the liver after delivery with glycoprotein carriers [123]. Another advantage of introducing phosphorylated nucleoside derivatives in the cell via covalent binding to carrier proteins may be an improved rate of cellular activation into the triphosphate form.

In general, caution is warranted in the synthesis of drug-protein complexes. Mild methods should be used that conserve the spatial conformation of the protein as much as possible. It should be taken into account that deviations from the normal charge distribution in the protein may lead to immunogenicity and loss of cell specificity. Prominent hydrophobic features of the connected drugs can lead to aggregation of the carrier molecules and capturing by phagocytotic systems. The higher the drug load, the more artefacts will be introduced with regard to cell specificity. In order to control such factors more easily, it has been advocated to replace the naturally occurring plasma-proteins with polypeptides carriers with a more simple structure.

2. AIDS and HIV

2.1 AIDS and HIV, an introduction

Human immunodeficiency virus (HIV) was first detected by Montagnier and coworkers in an electron micrograph in 1983 and identified as the cause of acquired immune deficiency syndrome (AIDS) in 1984. Two related but distinct viruses can

cause AIDS. HIV-1 is the virus isolated in 1983 and virtually all AIDS cases in Europe and the USA are associated with this type. HIV-2 was isolated in 1986, also by the group of Montagnier, from West-African AIDS patients. As the two viruses share very similar biological properties, most of what has been learned about HIV-1 appears to apply to HIV-2 also [134].

HIV is a retrovirus, which means that its genetic material is a ribonucleic acid (RNA) rather than a deoxyribonucleic acid (DNA). Retroviruses carry with them an enzyme called reverse transcriptase (RT) which catalyses transcription of viral RNA into double-helical DNA. This so called proviral DNA integrates into the genome of the host, resulting in an infection [135].

HIV belongs to the subgroup of lentiviruses. As the name implies, there are long periods of time between lentiviral infection and development of the disease. This may be due to an efficient immune control, to the ability of the infected cell to suppress virus replication or to an intrinsic viral property to slow down replication in the post initial stages of the infection [136]. The vehicle for HIV transmission includes peripheral blood, cell free plasma, cerebrospinal fluid, semen, breast milk, vaginal secretions, lung tissue, tears and saliva. However, only a limited number of routes appear to be common: sexual intercourse, exposure to infected blood or blood products and transmission from infected mothers to their infants *in utero* or by lactation after birth [137]. The main consequence of infection with HIV is a severe suppression of the immune system characterized by a major drop in the number of circulating CD4 positive T-lymphocytes accompanied by a broad range of opportunistic infections and cancers. Some individuals infected by the virus do not develop AIDS, but instead a condition called AIDS related complex (ARC) and suffer from fevers, diarrhoea and swollen lymph nodes [135].

Presently, HIV has infected more than 5 million people worldwide. Of these, roughly 50% live in Africa and 40% in North and South America [138]. It is estimated by WHO that the number of people world wide infected with HIV will be more than 10 million in the mid-1990s [139].

2.2 Human Immunodeficiency Virus (HIV)

Cellular targets of HIV

The identification of a retrovirus belonging to the family of the human T-cell leukaemia viruses [140,141] was rapidly followed by reports of the CD4 antigen being an essential component of the receptor for HIV [142,143]. The physiological role of these CD4 molecules is that of being adhesion molecules interacting with MHC class II molecules as well as playing a more overall active role in cell function [144]. Initially it was believed that the virus could only reproduce *in* vivo in CD4 positive T-lymphocytes [145], although evidence for a more extensive tropism was already emerging. Gartner [146] and Ho [147] simultaneously demonstrated that cells of mononuclear phagocytic origin could also be productively infected by different HIV isolates. Actually, cells belonging to the monocyte/macrophage (M/M) lineage are now supposed to play a crucial role in the pathogenesis and progression of the

disease [146,148,149].
The presence of CD4 molecules on endothelial cells and macrophages of the human hepatic sinusoids makes them putative targets for HIV and reservoirs of the virus. They may be involved in the pathogenesis of liver sinusoidal lesions and other abnormalities observed in AIDS patients [150,151]. Kupffer cells of human origin were shown to be sensitive for indirect infection through fusion with HIV positive lymphocytes. The virus multiplied abundantly and syncytium formation and virus budding was observed soon after infection [152,153].
Eosinophils are bone marrow derived granulocytic leucocytes and predominantly tissue dwelling cells. They localize to tissues that are in close contact with the external environment and are prominent in immunologic responses during allergic and helminthic parasitic diseases. The expression of the CD4 molecule [154] renders them possible target cells, which also holds for Langerhans cells, immunocompetent dendritic cells in the epidermis and stratified epithelia (oral, anal and vaginal mucosa, bladder epithelium, bronchial and cornea epithelium) that represent a subpopulation of the mononuclear phagocytic system [155,156].
The suggestion that CD4 is the only component essential for recognition/binding of HIV to its target cells, is rendered out of date now. Major Histocompatibility Complex class II appears to be involved in the binding, probably by virtue of its close proximity to the CD4 molecule [157]. In Langerhans cells it was shown that a second molecule exists on the cell membrane, which may act as an HIV receptor [155]. Glycosyl-phosphatidyl inositol (GPI) anchored membrane proteins also have the potential to serve as viral receptors [158]. With regard to cells of the nervous system, galactosyl ceramide or a molecule derived from it was suggested to be an alternative receptor for HIV [159].
Human T-lymphoid cells productively coinfected by HIV-1 and Human T-cell Leukaemia Virus type 1 (HTLV-I) or HTLV-II generated a progeny of phenotypically mixed viral particles. This phenotypic mixing resulted in penetration of HIV-1 into CD4 negative human cells, including mature CD8 positive T-lymphocytes, B-lymphoid cells, epithelial cells and skeletal muscle cells [160]. Furthermore, some human fibroblastoid cell lines can be infected by HIV independent of the CD4 protein expression [161].
Blocking the CD4 receptor of human monocytes with OKT4A monoclonal antibody did not prevent HIV-1 infection, again an observation favouring the presence of yet another HIV receptor. Penetration of HIV into CD4 negative cells was thought to be mediated by gp41. Gp41 bound to its 'receptor' on the cell membrane and virions were internalized via receptor mediated endocytosis [162].
This broad spectrum of HIV cellular tropism largely contributes to the pathogenicity of HIV and it is not difficult to see that it significantly hampers the chemotherapeutic interference with the infection.

Replicative cycle of HIV-1

The replicative cycle of HIV-1 (fig. 9) consists of various steps. First of all, the virus exhibits an interaction with its target cell by the envelope glycoprotein gp120. Whether or not this interaction requires the CD4 molecule, is discussed in the previous paragraph. The influence of the carbohydrate part of gp120 is a continuous subject of discussion. Early studies of Lifson and others [142,163-167] reported an important decrease in infectivity of HN-1 after removal of sugars or a change in

sugar composition of the gp120. In contrast, Fenouillet and coworkers [168] could not demonstrate a sugar dependent infection. Some groups have noted an important function for the carbohydrate chains in gp120 for cell recognition, but the question remains whether they were directly involved in the gp120-CD4 interaction or played a secondary role, e.g. through steric influences or multiple interactions with other membrane components [169-171]. On the other hand, the N-linked carbohydrates may also function as hindering structures, limiting recognition by the CD4 positive cells [172].

After recognition of the cell, the HIV associated envelope glycoprotein gp41 translocates into the membrane of the particular cell, inducing fusion of the membranes of virus and cell. In literature there is no consensus about the entry of the virus into the cell. Several authors hypothesized that entry merely consisted of membrane fusion and subsequent release of the viral genetic material (RNA) into the cytosol [173-175]. Using electron microscopy, among others, an endocytotic route of uptake was demonstrated [155,162,176,177]. One way or the other, the result of either route of entry is the cytosolic release of viral RNA and the enzyme reverse transcriptase. The conversion of viral RNA into double stranded DNA is carried out by this latter enzyme. After circularization, the viral DNA is integrated into the host cell genome. The HIV-1 genome encodes for a number of structural and functional proteins, which may be directly involved in one of the processes in the replicative cycle. Once activated, the viral DNA genome is transcribed to mRNA. Translation of the mRNA into viral proteins is followed by assembly and release ('budding') of new virus particles. Once infected, cells display the gp120 and gp41 entities on their membranes and are actively shedding gp120 molecules [178].

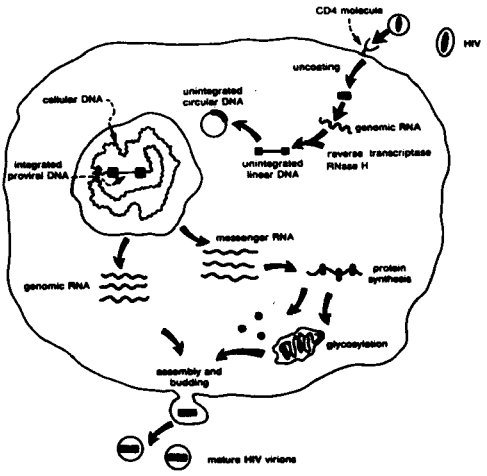


Figure 9. The replicative cycle of HIV-1 (from [179]).

So far, there is no clarity about the (exogenous and/or endogenous) signals converting a 'latent' infection with HIV-1 (in which the viral DNA is integrated in the host cell genome while viral RNA synthesis is absent or at least low-leveled [180]) into an acute infection. One cytopathic effect of an acute HIV-1 infection consists of fusion of infected cells, expressing the viral gp120 and gp41 glycoproteins in their membranes, with uninfected cells through a gp120-CD4 interaction. The resulting multinucleated giant cells (so called 'syncytia') will be destroyed by lysis. Experimental evidence, however, showed that other mechanisms of HIV induced cell killing were involved [181]. The cytopathic effect resulting in cell death and hence depletion of the CD4 positive T-lymphocyte subset, is responsible for the severe immunodeficiency characteristics of AIDS [182]. A chronic infection exists when normally dividing cells are infected in the absence of any cytopathic effect and persistently release infectious virus. The chronically or persistently infected cells (mainly monocytes and macrophages [147,183-185]) have been implicated as a reservoir of infectious virus, perhaps contributing to the slow progressive nature of HIV-related disease.

Influence of HIV-1 infection on host cells

HIV-1 induces acquired immune deficiency syndrome by pathogenic mechanisms which are not well understood to date. The virus directly infects and kills several cell types *in* vitro, still it is not clear if this is how the T-helper lymphocytes are depleted in *vivo*.

HIV is likely to affect different host cellular processes at different steps of its replicative cycle [186] and HIV envelope (env) gene products are thought to play a role in the cytopathology because the env gene products are membrane associated [186,187]. Another cytopathic event is syncytium formation. However, it occurs mainly in certain cell lines, at frequencies often not involving the majority of the cell population and rarely in cytopathic infections in peripheral blood lymphocytes grown in vitro.

HIV also inhibits the expression of IL-2 receptors on PHA-stimulated normal lymphocytes indicating a suppression during the early events of activation, probably due to a decreased inositol-phospholipid turnover [188]. Impairment of activation via the CD3/TcR phospholipase C transduction pathway may represent another suppressive mechanism [189].

Infection of two different human CD4 positive lymphoid cell lines (CEM and C8166) induced a markedly decrease in cellular protein synthesis at times when viral protein synthesis was increasing [190]. Other reported influences on host cell processes included suppression of IL-2 mRNA production [191], selective MHC II restricted antigen recognition defects in normal CD4 positive T-lymphocytes [192], an increase in intracellular levels of cAMP and cGMP (regulators of early events in T cell activation and antigen recognition) [193] and a significant increase in the molar ratio cholesterol/phospholipid of the plasma membrane of HIV infected human peripheral blood lymphocytes, thereby influencing cell membrane fluidity [194].

Monocytes from HIV-infected patients failed to undergo normal differentiation into macrophages *in* vitro [195] and autologous T-cells exerted an inhibitory effect on the *in* vitro growth of progenitor cells [196]. Lymphocyte 5'-ectonucleotidase activity, playing a role in maturation of T- and B-lymphocytes, was shown to be significantly lowered in AIDS patients [197].

It was estimated that during a cytocidal H N infection the virus used nearly all of the cells' capacity to make RNA and proteins. However, most cells susceptible to **HIV cytopathology** produce HTV **RNA** and proteins in quantities similar to those made by other retroviruses which are not cytopathic **[186].**
Although there is no experimental evidence on the direct infection of endothelial cells by HN, they are involved in many cytopathic situations and the immune function of vascular endothelium was recently shown to be impaired in **HIV** infection **[198].**

2.3 Chemotherapeutic approach of HIV-1 infections

The replicative cycle of HIV-1 offers a wealth of possible targets for antiviral agents. The virus adsorption process can be inhibited by ***polyanionic substances*** such as sulfated polymers (dextran sulfate, pentosan sulfate, heparin **[178,199-201], polyvinylalcohol** sulfate (PVAS) and polyacrylicacid-vinylalcohol sulfate (PAVAS) **[201])** and polyanions, such as **aurintricarboxylic** acid (ATA) **[201-203]** and *CD4* constructs **[204-206].** The sulfated polymers act by shielding off the viral **gp120,** whereas ATA predominantly interacts with the **CD4** receptor **[201].** The **CD4** constructs competitively inhibit viral binding to the cellular CD4. It was recently demonstrated that **HIV** inactivation by soluble **CD4** was also accomplished due to the release of membrane associated **gp120** from infected cells, thereby inhibiting **syncytium** formation **[207].** *Lectins* recognizing specific sugar moieties of the viral **gp120** are able to shield this glycoprotein from recognizing the target cells **[208]** and negatively ***charged*** albumins were recently shown by us to be active against HIV induced **cytopathicity** by inhibition of the fusion of virus and cell after recognition and absorption **[209].** The **polyanionic** substances and **CD4** constructs also protect the CD4 positive cells against killing by **gp120** positive cells **[201].**
Nucleoside analogues inhibit **HIV-1** replication through two different mechanisms. Only after conversion to their active triphosphate derivatives by cellular enzymes, they are able to interact with their target enzyme reverse transcriptase (RT). This strategy seems very attractive because it attacks a step that is unique to retroviruses. The compounds can function, in their phosphorylated form, as either competitive inhibitors of RT or as an alternative substrate with respect to the natural substrate thymidine-triphosphate **[201].** If acting as a substrate, the triphosphates are incorporated into the DNA chain, leading to chain termination due to the lack of the 3'-hydroxyl group. Examples of these nucleoside analogues are the 2',3'-dideoxynucleosides **ddC [210,211]** and **ddI [212,213]** and the **azido** congener AZT **[214,215].** Recently a new class of antiviral agents, based on the non-nucleoside compound **tetrahydro-imidazo [4,5,1-jk][1,4]-benzodiazepin-2(1H)-(thi)one** (TIBO) was described. They were shown to be potent and selective **anti-HIV-1** agents in ***vitro,*** also acting as inhibitors of the reverse transcriptase **[216,217].**
The next target for therapy presents itself some time later in the cycle of **HIV,** at the transcription and translation steps of viral DNA into viral protein. Using so called antisense ***nucleotides,*** the translation of the HIV genome can be arrested. The antisense constructs are complementary to a part of the viral **mRNA** and can bind to these parts. Through hybridization, the cell's ribosomes are obstructed from moving

along the RNA, thereby halting the translation of RNA into viral protein [218]. So far, several antisense nucleotides and analogs have been reported as potent anti-HIV agents [19,219,220].

After the viral proteins are produced, carbohydrates are enzymatically added in the glycosylation process. Protein N-glycosylation is critical to the pathogenesis of HIV-1 at the level of viral infectivity and cytopathicity [221]. The *glucosidase inhibitors* castanospermine and 1-deoxynojirimycin show an inhibitory effect on the expression of the HIV-env glycoprotein, thereby blocking syncytium formation and interfering with infectivity [163,222,223].

The virus encoded protease, essential for the cleavage of viral polyproteins into structural proteins of the virion core and enzymes essential for retrovirus replication, is a potentially ideal target for antiviral therapy. The *protease* is sufficiently distinct from cellular proteinases to allow its preferential *inhibition* [179], e.g. by synthetic peptide analogues [224,225] or butyrophenone derivatives [226].

Finally, assembly and budding of virus particles can be stopped by *interferons*. In addition to being used in the chemotherapeutic approach of HIV infections, interferons are naturally produced in cells and are thought to act at other steps in the HIV life cycle as well [218,227].

The HIV-induced giant cell formation (syncytium formation) is potently inhibited by D-mannose specific plant *lectins* [208]. This inhibition is assumed to be due to an interaction directly with the virus-cell membrane fusion process. Hansen [228] reported on blockage of several glycan structures on gp120 by various lectins, also resulting in inhibition of CD4-gp120 induced cell fusion.

3. Lymphocytes, monocytes and macrophages

3.1 Receptors on T and B lymphocytes, monocytes and macrophages

To accomplish cell specific targeting of drugs using macromolecular carriers, more insight in the presence of all kinds of receptors on the various target cell types is desirable. Furthermore, knowledge about processes occurring after recognition/ interaction of the specific ligand with its receptor may offer a rationale for estimating the possibilities of establishing intracellular delivery and release of the ligand associated drug.

3.2 Receptors for endogenous ligands

T lymphocytes become activated in the presence of *Interleukin-I* (IL-1) or Interleukin-6 (IL-6) after recognition of a complex of antigen and major histocompatibility complex (MHC) encoded molecules on the surface of B cells and macrophages [229]. Although murine IL-1 and human IL-1 a and ß exhibit differences in amino acid sequence, they all bind to the same receptor site on various

murine T-cell lines [230]. When murine T-cell lymphoma and a murine fibroblast cell line were incubated in the presence of IL-1, the IL-1 receptor (IL-1R) underwent extensive ligand-induced downregulation at 37 °C. IL-1 was internalized, a requirement for its activity [231], but did not undergo degradation for at least 6 hrs. Relatively high amounts of IL-1 accumulated in the cell nucleus [230].

Activated T-cells produce Interleukin-2 and simultaneously express the high affinity IL-2 receptor (IL-2R) [229]. IL-2 activation of CD8 positive T-cells results in augmented cytotoxicity against natural killer cell sensitive and non-sensitive target cells [232]. After binding to its receptor, IL-2 is internalized by receptor mediated endocytosis (RME) and via endosomal compartments delivered to lysosomes for degradation [233]. In a human tumour T cell line (IARC 301), IL-2R were constantly internalized and not recycled after RME [234]. In PHA stimulated human peripheral blood lymphocytes two IL-2R pools were identified and, as in the IARC 301 cells, retroendocytosis did not occur to a significant extent [235].

Interleukin-4 is secreted by activated T-lymphocytes and exhibits diverse biological activities. Human peripheral blood mononuclear cells (PBMC) express ~300 high affinity IL-4 receptors per cell, while in *vitro* PHA or ConA activated PBMC increased this amount 2 to 4.5 fold, without alteration of IL-4 affinity. A low level of expression also exists on various cell lines (<3000 IL-4R per cell) [236]. Human monocytes and acute myeloblastic leukaemic cells contain ~200 and 100 high affinity and 1500 and 2400 low affinity IL-4 binding sites, respectively [237].

Interleukin-6 is a cytokine able to induce acute-phase protein synthesis. This induction is mediated by a direct signal through IL-6 receptors on the surface of hepatocytes. IL-6 also augments cytotoxic T-cell generation and B-cell Ig secretion [238]. It exists in different molecular isoforms, due to various glycosylation patterns [239]. At 37 °C, IL-6 was rapidly internalized by human T-cells and monocytes/ macrophages, and, after a lag time, degraded moieties were released from the cells [238].

α2-Macroglobulin, a homologous plasma proteinase inhibitor, forms stable complexes with a wide spectrum of different proteolytic enzymes. The complex formation induces a conformational change leading to the exposure of receptor recognition sites. A common plasma membrane receptor with high affinity for α2-macroglobulin-proteinase complex has been described in human monocytes and macrophages, among others. It was demonstrated that receptor-mediated endocytosis and lysosomal degradation of the complex took place. 1500-2000 α2-macroglobulin-proteinase binding sites per human blood monocyte were estimated to be present [240].

Transferrin (Tf) is an endogenous glycoprotein, in which the glycans can differ in their degree of branching (bi-, tri- or tetraantennary) depending on the source of the macromolecule [241]. Transferrin receptors are present on those cell types actively dividing [242], implicating its iron-transport and -delivery function in growth and differentiation of a variety of cells [241]. During activation, T-cells internalize and recycle their Tf receptors [242].

The main biological function of ***alpha-fetoprotein*** (AFP) is the regulation of delivery of fatty acids to actively dividing cells through receptor mediated endocytosis. Mitogen stimulated normal human lymphocytes bind and internalize AFP, as do human T and B neoplastic lymphoid cells [243].

Ageing of long-lived extracellular proteins is accompanied by conjugation with

glucose, resulting in so called advanced ***glycosylation*** end proteins (AGE-proteins) [244]. In ***vitro***, AGE modified bovine serum albumin (BSA) specifically bound to mouse peritoneal **macrophages** and was taken up and degraded at 37 °C. These processes were concentration dependent and saturable. Approximately 10^5 receptors per macrophage were present, and they showed distinct characteristics from other scavenger receptors **[245,246].** Recently, two different AGE protein binding proteins, expressed on rat monocytes and resident peritoneal macrophages, were isolated. These 60 and 90 kD membrane proteins were suggested to be related to the above described receptor **[247].**

Human and mouse T-lymphocytes contain carbohydrates, usually expressed as **gangliosides,** that can act as cell surface receptors for ***regulatory molecules***, e.g. interferon, thyroid stimulating hormone, and for ***immunoregulatory*** molecules, e.g. factors mediating help and suppression of antibody formation [248]. Human monocytes and alveolar macrophages express insulin like growth-factor **(IGF)-II/mannose-6-phosphate** receptors, 10,000 and 20,000 receptors per cell, respectively **[249].** Upon a-CD3 activation, human peripheral blood T-cells demonstrated the sequential appearance of receptors for IGF-I, IGF-11 and insulin **[250].**

Up to 60% of resting blood T-lymphocytes and **monocytes/macrophages** from **normal** subjects show evidence of spontaneous internalization of ***MHC class I molecules*** via coated pits **[242,251].** For the endocytosis of gamma-endorphin **co-endocytosis** of **MHC** I was essential, suggesting that **MHC** I molecules may function as transport molecules [251]. Activation of T-lymphocytes by allogenic cells or **lectins** increased the number and size of vesicles with MHC class I molecules. In addition, ***MHC class II*** molecules were shown to be present in a comparable number of intracytoplasmic vesicles [242].

β-Glucans are the major structural components of yeast and fungi and contain 98% glucose. Receptors for these particulate immune activators are present on human monocytes and initiate phagocytosis of glucan, production of **leukotrienes** and release of lysosomal enzymes. The receptors only recognize **ß-glucans,** not **α-glucans, mannan** or **galactan [252,253].**

Although ***gp120*** is not an endogenous compound, it should be mentioned here due to the fact that in AIDS patients it is shedded from infected cells into the blood. **Lanzavecchia** showed that activated MHC class **II** positive T-lymphocytes were capable of internalizing, processing and presenting this antigen **[254].**

In conclusion, it can be said that those blood cell types, that serve as target cells for **HIV-1,** are capable of recognizing specific ligands (summarized in table III). Furthermore, a number of ligands can be endocytosed and degraded, indicating that the cellular machinery needed for these processes, is in principle present.

3.3 Membrane lectins

Lectins are sugar binding proteins of non immune origin which agglutinate cells and/or precipitate **glycoconjugates.** They are found in nature in a wide variety of bacteria, plants, invertebrates and vertebrates **[255].** Most lectins interact specifically with a simple sugar although for some the specificity is broader and includes closely related sugars. Certain lectins only interact with complex carbohydrate structures

[256].
Several cellular processes in mammals have now been attributed to lectin-mediated recognition events. Lectins on lymphocytes are involved in the first stage of recirculation from the bloodstream into lymphoid tissue through an interaction with so called high endothelial venules (HEV) [257]. The expression of lectins on lymphocytes, monocytes and macrophages and upregulation upon activation suggests a potential role in immune responses [258-260]. The presence of lectins in endosomes, lysosomes and even in the nucleus suggests intracellular functions for these sugar receptors [261-263]. Various kinds of extracellular ligands such as hormones, growth factors, antibodies, viruses and toxins are taken up into cells by lectin-receptor mediated endocytosis [263].

Table III. Endogenous ligands and their target cells.

Endogenous ligand	Cell type	Reference
IL-1, α/ß	murine T-cell lines	[230,231]
	fibroblast cell lines	
IL-2	activated T-cells	[229]
	activated human PBLs	[235]
	human tumour T-cells (IARC 301)	[234]
IL-4	PBMC	[236]
	various cell lines	[236]
	human monocytes	[237]
	human acute myeloblastic cells	[237]
IL-6	hepatocytes	[238]
	human T-cells, monocytes, macrophages	
α2-macroglobulin	human monocytes, macrophages	[240]
transferrin	actively dividing cells	[242]
α-fetoprotein	mitogen stimulated human lymphocytes	[243]
	human T and B neoplastic lymphoid cells	
AGE-proteins	mouse peritoneal macrophages	[245,246]
	rat monocytes and peritoneal macrophages	[247]
(immuno)regulatory molecules	human/mouse T-lymphocytes	[248]
IGF-II/mannose-6-phosphate	human monocytes	[249]
	human alveolar macrophages	
IGF-I/IGF-II/insulin	a-CD3 activated human T-lymphocytes	[250]
MHC class I	resting human T-lymphocytes, monocytes, macrophages	[242,251]
	activated human T-lymphocytes	[242]
MHC class II	activated human T-lymphocytes	[242]
ß-glucans	human monocytes	[252,253]
gp120	MHC class II positive T-lymphocytes	[254]

While the role of mammalian lectins is very diverse, the specificity of sugar recognition may be even more diverse. Factors influencing specific recognition by and

affinity for lectins are :

1) cell type under investigation
2) species origin of the cells
3) structure complexicity of the carbohydrate chains [119,264-266]
4) density of the sugars [266-269]
5) lectin environment (e.g. solubilized in blood or tissue fluid vs. cell membrane incorporated) [266].

Cells of human origin.

The process of binding of bacteria to human lymphocytes is mediated by lectins present on the lymphocyte. Binding can be partially blocked by α-methyl-D-mannopyranoside [270], D-glucose or L-fucose, dependent on the type of bacteria under investigation [260].

Recently, a ß-galactoside specific lectin was isolated from human B and T lymphoblastoid cells. The lectin was purified using affinity chromatography with Biogel A15M immobilized fetuin. Interestingly, only high concentrations of &lactose, but not galactose, galNAc, mannose, glucose, glucNAc and xylose, resulted in elution of the lectin from the column [271].

Suppression of a typical immune response could be inhibited by a-L-rhamnose, specific for a lectin on human CD8 positive T-cell rich population [272].

Agglutination of human peripheral blood lymphocytes using mannosyl-thiocarbamyl BSA implicated the presence of a mannose specific lectin on these cells. After fractionation of the total blood cell population, B-lymphocytes were identified to possess the lectin, not T-lymphocytes [273].

Human mononuclear phagocytes express a unique cell surface receptor that specifically recognizes glycoproteins bearing terminal mannose, fucose and glucNAc residues. The mannose receptor binds and internalizes ligands by receptor mediated endocytosis. In monocyte derived macrophages of human origin, the receptor exhibits a half-life of 33 hrs, indicating that each receptor molecule recycles hundreds of times between the cell surface and endosomes before degradation [274]. Expression of the receptor is closely regulated. In contrast to these mannose receptors, which appear only after maturation, mannose-6-phosphate receptors are already present on freshly isolated human monocytes [275]. Likewise, the receptor expression changes according to the macrophage type and downregulation occurs upon macrophage activation. Most macrophage cell lines are devoid of cell surface mannose receptors [267,276]. Recently, a D-mannose specific receptor was isolated from the human promyelocytic cell line HL60 [276]. On human circulating polymorphonuclear cells (granulocytes), receptors specific for α-L-rhamnosyl residues were demonstrated (–55,000 receptors per cell) [277].

Cells of murine origin.

At least three lectin specificities are present on mouse spleen and thymus lymphocytes. Lectins specific towards ß-D-galactosides and N-Acetyl-D-galactosamine are mainly present in the lymphocytes originating from thymus, whereas a-D-mannoside specificity is present on spleen lymphocytes [278,279].

Two mannose-binding proteins on unstimulated and three other mannose-binding proteins on *in* vitro stimulated spleen lymphocytes were more recently described [280].

The a-globulin fetuin, present in fetal calf serum, has complex branched oligosaccharide chains with terminal sialic acid residues linked via galactose and N-Acetyl-glucosamine (glucNAc) to a mannose and glucNAc backbone. It binds to mouse thymus and spleen cells and this binding is strongly inhibited by asialofetuin, suggesting a role for galactose recognizing lectins [264].

The binding of autologous erythrocytes to lymphocytes, a phenomenon termed autorosetting, involves recognition of 'self' cells. The murin autorosetting receptor is a protein that recognizes the carbohydrate portion of a glycoprotein molecule on the erythrocytes. Further examination of this autorosetting receptor revealed the primarily recognition of sulphated polysaccharides [281]. Other cell types (macrophages, polymorphonuclear leucocytes, mast cells and fibroblasts) were also able to bind similar polysaccharides. However, each cell type bound a characteristic array of polysaccharides. Whereas heparin, fucoidan and k-carrageenan were specifically endocytosed by macrophages, the other polysaccharides were not taken up by any of the cell types examined. A functional role in a range of cell-cell interactions was suggested for this sulphated polysaccharide recognition [282].

Macrophages of murine origin bound and internalized mannosylated and 6-phosphomannosylated ligands, whereas freshly isolated monocytes only expressed the mannose-6-phosphate membrane lectin [65].

Comparison of the internalization of Con A reactive al-acid glycoprotein (AGP) variants by a mouse macrophage hybrid and normal mouse macrophages, revealed that the AGP variant containing two biantennary structures was better internalized by both types of cells than those bearing one or no biantennary structures. It was demonstrated, that the intracellular half life of the internalized ligands and the degrading capacity were quite different in the two types of macrophages [267].

On the cell surface of mouse L1210 leukaemia cells, the presence of a fucose-specific lectin was demonstrated. The ligand with highest affinity was BSA substituted with 25 fucose residues. An active membrane lectin-mediated endocytosis was suggested [283].

Galactose-specific lectin expression on the surface of mouse mast cells and macrophages (designated IgE-binding protein) was quite variable among the various cell lines studied. The number of lectin molecules per cell varied between 1,000 and 125,000 [284].

Cells of rat origin.

Recognition and plasma clearance of various glycoproteins and enzymes are mediated by cell surface receptors associated with liver sinusoidal cells and other cells of the mononuclear phagocyte system. Using rat and rabbit alveolar macrophages, L-fucose-albumin was shown to be taken up into the macrophages by receptor mediated endocytosis, whereas D-fucose-albumin was poorly internalized. Inhibition experiments indicated that the macrophage receptor had broad specificity and was able to bind various glycoconjugates, most avidly however those expressing terminal L-fucose or D-mannose, suggesting one 'lectin-receptor' for both sugars [285,286a].

Rat liver Kupffer cells were shown to possess a sugar specific receptor for galactose containing particles [286b].

Rat alveolar macrophages have one receptor for mannose, glucNAc or glucose in the terminal, non-reducing position. This recognition is restricted to macrophages and apparently not expressed on polymorphonuclear leucocytes or lymphocytes [287].

Macrophages of peritoneal origin possess a galactose specific recognizing system [266].
Lymphocyte cell surface lectins specific for fucose and related structures possibly contribute to the specific attachment of lymphocytes to postcapillary venules [257].

Cells of porcine origin.
The importance of oligosaccharide branching for recognition of the glycoprotein was suggested by Bezouska. Using rat and pig hepatocytes and pig T and B lymphocytes and mononuclear cells, it was shown that hepatocytes and polymorphonuclear cells specifically bound the more branched (galactose terminating) asialo-oligosaccharides, lymphocytes specifically the less branched and macrophages all structures to the same extent. The number of binding sites per cell varied with the cell type [265]. Experiments with asialoglycoproteins having different oligosaccharide branching affirmed the above described specificity. It was also shown that the number of oligosaccharide chains per glycoprotein molecule is less important [119]. A summary of the sugar specific lectin distribution on blood cells is given in table IV.
Although the above described sugar-specific interactions of various compounds with lectins on different cell types seem to be merely restricted to a few types of sugars, it is more likely that binding involves a combination of sugars. The use of one sugar type in inhibition experiments, as described by various authors, may be sufficient to prevent binding of the spatially arranged sugars by steric hindrance, it may also give an unrealistic image of the real recognition sites.

Table IV. Distribution of sugar specific lectins on several blood cell types and cell lines in different species

species	cell type	sugar specificity	reference
human	lymphocytes	mannose	[270]
		mannose,glucose,fucose	[260]
		galactose	[271,288]
	B-lymphocytes	mannose	[273]
	mononuclear phagocytes	mannose,fucose,glucNAc	[274]
	monocytes	mannose-6-phosphate	[275]
	macrophages	mannose,mannose-6-phosphate	[275]
	promyelocytic cell HL60	mannose	[276]
	granulocytes	rhamnose	[277]
mouse	lymphocytes, splenic	mannose	[278-280]
	lymphocytes, thymic	galactose,galNAc	[264,278,279]
	lymphocytes	sulphated polysaccharides	[281]
	macrophages	heparin,fucoidan,	
		k-carrageenan	[282]
		mannose,mannose-6-phosphate	[65,267]
		galactose	[284]
	monocytes	mannose-6-phosphate	[65]
	L1210 leukaemia cells	fucose	[283]
	mast cells	galactose	[284]
rat	macrophages, alveolar	L-fucose,mannose,glucNAc,	
		glucose	[285-287]
	Kupffer cells	galactose (particle)	[286b]
	macrophages, peritoneal	galactose	[266]
	lymphocytes	fucose	[257]
pig	lymphocytes	galactose	[119,265]
	mononuclear cells	galactose	[119,265]

Lymphocyte homing receptors.
Homing receptors are lectin-like receptors on the cell membrane surface of various lymphocyte subsets [289], functionally indispensable to the recirculation of lymphocytes from the blood to lymphoid organs [257]. High endothelial venules (HEV) express tissue specific carbohydrate containing determinants for lymphocyte recognition and so far, at least three distinct lymphocyte-HEV recognition systems have been characterized [290]. Determinants specifically recognized by these lectins consist of a mannose, fructose or fucose moiety and some negative charge, e.g. mannose-6-phosphate [289,291-294], fructose-1-phosphate [289,294], fucose-4-sulphate [292], fucoidin [257,291,294] and phosphomannan [293,294]. Lymphocyte homing receptors are believed to belong to the family of lectin-like proteins, which includes the serum mannose-binding protein and the mannose receptor on various tissue macrophages. Among these homing receptors, the mannose receptor is thought to be the only member that can mediate phagocytosis [295]. Therefore, homing receptor lectins on lymphocytes can probably only serve to mediate the extracellular release of drugs bound to suitable carriers.

4. Aim of the study

To date, the most commonly used drug for the treatment of AIDS and ARC patients is the nucleoside analogue AZT (3'-azido-3'-deoxythymidine, Retrovir^{R}, zidovudine). Early phase treatment with AZT increases the number of circulating CD4 positive lymphocytes and appears to be associated with temporarily clinical improvement and decreased mortality and frequency of opportunistic infections in some patients [214,296].
However, the drug has several disadvantages. First, anaemia, leucopenia and neutropenia are the major haematologic abnormalities attributable to AZT and found in a majority of subjects receiving the drug [296]. Second, isolates of HIV from patients who are treated with AZT for approximately 6 months or longer, frequently develop resistance towards the drug [297]. Looking at the pharmacokinetic characteristics of the drug in man, a non-optimal profile in relation to the therapeutic aim is obtained. The drug is rapidly cleared from the body (plasma half life in man is ~1-2 hrs) by both renal excretion and metabolism to 5'-glucuronylazidothymidine [298].
The above described disadvantages can in principal be overcome by specifically delivering the drug (in)to those cell types in which the drug should exhibit its anti-HIV activity. The main target cells for AZT, therefore, are those cell types in which HIV-1 most avidly replicates, i.e. the CD4 positive T-lymphocytes. Although the number of CD4 positive T-lymphocytes infected by HIV-I was first thought to be very low, it is now well accepted that as much as 1 in 100 circulating lymphocytes contains proviral DNA in various stages of the disease [299].
The aim of the study presented in this thesis was to investigate the possibilities of specific targeting of AZT to the helper/inducer subset of T-lymphocytes using neoglycoproteins (albumin chemically modified with sugar derivatives) as carrier molecules, thereby eliminating the major toxicity and kinetic disadvantages and

increasing the therapeutic efficacy of the drug.
The resulting research included the synthesis of neoglycoproteins and their AZT conjugates and development of methods for the analysis of physico-chemical properties of the conjugates (chapter 2). The anti-HIV-1/2 activities *in vitro* in MT-4 cells of neoglycoproteins and conjugates were determined and mechanisms of antiviral activity were studied (chapters 3, 6 and 8). Several cell types commonly used for the determination of anti-HIV activity of all kinds of compounds and for studying cytopathogenicity of the virus were compared with respect to binding and uptake characteristics of various neoglycoproteins and conjugates, in order to find an appropriate cell model for in *vitro* determination of pharmacokinetic features of neoglycoprotein-drug conjugates in general (chapter 7). The synthesis of AZT conjugates was optimised, resulting in products with low polymerisation degree, low clearance by the rat liver cells and higher stability upon storage (chapter 5), all factors being a major requirement for future *in vivo* use in animal models and patient studies. Development of a simple and rapid HPLC method for the simultaneous analysis of AZT and its mono-, di- and triphosphate derivatives and an extraction method for all compounds (chapter 4) offered the opportunity to perform *in vitro* kinetic studies using AZT, AZTMP and the conjugates and will be useful in further studying the *in vivo* behaviour of drugs and conjugates.

References

1. Boddy AV and Aarons LJ, Application of a linear recirculation model to drugtargeting. J *Pharmacokinet Biopharm* 19: 355-362, 1991.
2. Siegel RA, MacGregor RD and Hunt CA, Comparison and critique of two models for regional drug delivery. J *Pharmacokinet Biopharm* 19: 363-373, 1991.
3. Stella VJ, Himmelstein KJ, Prodrugs: A chemical approach to targeted drug delivery. In: *Directed drug delivery. A multidisciplinary problem* (Eds. Borchardt RT, Repta AJ and Stella VJ), pp. 247-267. Humana Press, Clifton, New Jersey, 1985.
   Storm G, Peeters P, Nässander UK, Crommelin DJA, Drug targeting with immunoliposomes. In: *Trends in drug research* (Eds. Claassen V and Timmerman H), pp. 333-349. Elseviers Scientific Publisher, Amsterdam, 1990.
   Friden PM, Walus LR, Musso GF, Taylor MA, Malfroy B and Starzyk RM, Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. *Proc Natl Acad Sci USA 88:* 4771-4775, 1991.
6. Umezawa F and Eto Y, Liposome targeting to mouse brain: mannose as a recognition marker. *Biochem Biophys Res Commun* 153: 1038-1044, 1988.
7. Tomlinson E, Site-specific drugs and delivery systems: rationale, potential and limitations. In: *Trends in Drug Research* (Ed. Claassen V), pp. 287-302. Elsevier, Amsterdam, 1990.
8. Poznansky MJ and Juliano RL, Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol Rev* 36: 277-336, 1984.
9. Meijer DKF, Jansen RW, Molema G, Disposition of Macromolecules in the Liver in Relation to Drug Targeting. In: *Current Topics in Gastroenterology and Hepatdogy* (Eds. Tytgat GNJ and Van Blankenstein M), pp. 374-392. Georg Thieme Verlag, Stuttgart, 1990.
10. Meijer DKF, Molema G, Jansen RW, Moolenaar F, Design of cell-s c drug targeting preparations for the liver: where cell biology and medicinal chemistry meet. In: *Trends in drug research, proceedings of the seventh Noordwijkerhout-Camerino Symposium* (Eds. Timmerman *H* and Claassen V), pp. 303-332. Elsevier, Amsterdam, 1990.
11. Cretton EM, Xie M-Y, Bevan RJ, Goudgaon NM, Schinazi RF and Sommadossi JP,

Catabolism of 3'-Azido-3'-Deoxythymidine in **Hepatocytes and** Liver **Microsomes,** with Evidence of Formation of **3'-Amino-3'-Deoxythymidine,** a Highly Toxic **Catabolite** for Human **Bone** Marrow Cells. ***Mol Pharmacol 39:*** **258-266,1991.**

12. Canonico PG, **Kende** M and **Gabrielsen** B, Carrier-mediated delivery of antiviral **agents.** ***Adv Virus Res 35:*** **271-312,1988.**
13. Barchi **JJ,Jr.,** Marquez VE, Driscoll **JS,** Ford **H,Jr., Mitsuya** H, **Shirasaka** T, **Aoki S** and **Kelley** JA, Potential Anti-AIDS Drugs. **Lipophilic,** Adenosine **Deaminase-Activated Prodrugs.** J ***Med Chem*** **34: 1647-1655,1991.**
14. **Steim** JM, Neto CC, Sarin PS, Sun DK, Sehgal RK and Turcotte JG, Lipid Conjugates of **Antiretroviral** Agents .I. Azidothymidine-Monophosphate-Diglyceride: Anti-HIV Activity, Physical Properties, **and** Interaction with Plasma Proteins. ***Biochem Biophys Res Commun*** 171: **451-457, 1990.**
15. **Piantadosi** C, **Marasco CJ,Jr., Morris-Natschke** SL, Meyer KL, Gumus F, **Surles** JR, Ishaq KS, Kucera LS, Iyer N, **Wallen** CA, Piantadosi **S and** Modest EJ, Synthesis **and** Evaluation **of** Novel Ether Lipid Nucleoside Conjugates for **Anti-HIV-1** Activity. ***J Med Chem*** **34: 1408-1414, 1991.**
16. **Hostetler KY and** Richman DD, **Inhibition** of HIV replication by novel phospholipid derivatives of **AZT and** other **antiretroviral** agents in **vitro.** ***5th Int AIDS Conference*** **june,8: 1989.(Abstract)**
17. MacCoss M, Edwards JJ, Lagocki P and Rahman YE, **Phospholipid-nucleoside conjugates. 5.** The interaction of selected 1-ß-D-arabinofuranosylcytosine-5'-diphosphate-**L-1,2-diacyl-glycerols** with serum lipoproteins. ***Biochem Biophys Res Commun*** 116: **368-374, 1983.**
18. Welch **CJ, Larsson A,** Ericson AC, Oberg B, **Datema** R and **Chattopadhyaya** J, **The** chemical synthesis and **antiviral** properties of an acyclovir-phospholipid conjugate. ***Acta Chem Scand 39:*** **47-54, 1985.**
19. Stein CA, **Matsukura** M, **Subasinghe** C, **Broder S** and **Cohen** JS, **Phosphorothioate Oligodeoxynucleotides** Are Potent Sequence **Nonspecific** Inhibitors of De Novo Infection by **HIV.** ***AIDS Res Hum Retroviruses 5:*** **639-646, 1989.**
20. Neto CC, **Steim** JM, Sarin PS, Sun DK, **Bhongle** NN, **Piratla** RK **and** Turcotte JG, Lipid Conjugates of **Antiretroviral** Agents. II. **Disodium Palmityl Phosphonoformate:** Anti-HIV Activity, Physical Properties, and Interaction with Plasma **Proteins.** ***Biochem Biophys Res Commun*** 171: **458–464,1990.**
21. **Bodor** N, Chemical ***drug*** delivery systems. In: ***Bioreversible carriers on drug design. Theory and application (Ed.*** **Roche EB),** pp. **95-120.** Pergamon **Press,** New York, **1989.**
22. **Chu** CK, **Bhadti** VS, Doshi KJ, Etse JT, **Gallo** JM, **Boudinot** FD and Schinazi RF, Brain Targeting of Anti-HIV **Nucleosides:** Synthesis and In **Vitro** and In **Vivo Studies** of **Dihydropyridine** Derivatives of **3'-Azido-2',3'-Dideoxyuridine** and **3'-Azido-3'-Deoxy-thymidine.** J ***Med Chem 33:*** **2188-2192, 1990.**
23. **Bijsterbosch** MK **and** Van **Berkel** TJC, Native and modified lipoproteins as drug delivery systems. ***Adv Drug Deliv Rev 5:*** **231-251, 1990.**
24. Szoka **FC,Jr.,** The Future of **Liposomal** Drug Delivery. ***Biotechnol Appl Biochem*** 12: **4%-500,1990.**
25. **Gregoriadis** G, Targeting of drugs with molecules, cells and **liposomes.** ***Trends Pharmacol Sci 4:*** **304-307,1983.**
26. **Weinstein** JN and Leserman LD, **Liposomes** as ***drug*** **carriers** in cancer chemotherapy. ***Pharmacol Ther*** **24: 207-233,1984.**
27. Sullivan SM, Connor J and Huang L, **Immunoliposomes:** preparation, properties and applications. ***Med Res*** Rev ***6:*** **171-195, 1986.**
28. **Crommelin** DJA, Storm G, Review ***of*** progress in ***drug*** targeting. In: ***Comprehensive Medicinal Chemistry*** (Ed. Taylor JD), pp. **1-89.** Pergamon, Oxford, **1988.**
29. Allen TM, Toxicity of drug carriers to the **mononuclear phagocyte** system. ***Adv Drug Deliv Rev*** **2: 55-67, 1988.**
30. **Gabizon** A **and** Papahadjopoulos D, **Liposome** formulations with prolonged circulation time in blood **and** enhanced uptake by tumors. ***Proc Natl Acad Sci USA 85:*** **6949-6953, 1988.**

31a. Papahadjopoulos D, Gabizon A, Liposome targeting to tumor cells in vivo. In: *Targeting of drugs 2. Optimization strategies* (Eds. Gregoriadis G, Allison AC and Poste G), pp. 95-101. Plenum Press, NY/London, 1990.

31b. Scherphof GL, In vivo behavior of liposomes: interactions with the mononuclear phagocyte system and implications for drug targeting. *Handbook of Experimental Pharmacology* vol. 100 *(Ed.* Juliano RL), Springer-Verlag, Berlin/Heidelberg, pp.285-327, 1991

32. Phillips NC, Skamene E and Tsoukas C, Liposomal encapsulation of 3'-ado-3'-deoxythymidine (AZT) results in decreased bone marrow toxicity and enhanced activity against murine AIDS-induced immunosuppression, *J* Acquir *Immune Defic Syndr 4:* 959-966, 1991.

33. Bakker-Woudenberg IAJM, Lokerse AF and Ten Kate M, Increased Efficacy of Ganciclovir and Foscarnet Inhibition of Cytomegalovirus Replication In Vitro by Encapsulation in Liposomes. *Scand J Infect Dis* 74: 54-57, 1991.

34. Roerdink FH, Daemen T, Bakker-Woudenberg IAJM, Horm G, Crommelin DJA, Scherphof GL, Therapeutic utility of liposomes. In: *Drug* Delivery *System, Fundamentals and Techniques* (Eds. Johnson P and Lloyd-Jones JG), pp. 66-80. VCH, 1988.

35. Lazdins JK, Woods-Cook K, Walker M and Alteri E, The Lipophilic Muramyl Peptide MTP-PE Is a Potent Inhibitor of HIV Replication in Macrophages. AIDS *Res* Hum *Retroviruses 6:* 1157-1161, 1990.

36. Konopka K, Davis BR, Larsen CE, Alford DR, Debs RJ and Düzgünes N, Liposomes modulate human immunodeficiency virus infectivity. J *Gen Virol* 71: 2899-2907, 1990.

37. De Clercq E, Targets and strategies for the antiviral chemotherapy of AIDS. *Trends Pharmacol Sci* 11: 1%205, 1990.

38. Leonetti J-P, Machy P, Degols G, Lebleu B and Leserman L, Antibody-targeted liposomes containing oligodeoxyribonucleotides complementary to viral RNA selectively inhibit viral replication. *Proc Natl Acad Sci USA 87:* 2448-2451, 1990.

39. Milhaud PG, Machy P, Lebleu B and Leserman L, Antibody targeted liposomes containing poly(rI).poly(rC) exert a specific antiviral and toxic effect on cells primed with interferons α/β or r. *Biochim Biophys Acta* 987: 15-20, 1989.

40. Machy P, Leserman L, 'Piloting' of drugs and biological macromolecules: approaches to therapy. In: *Liposomes in cell biology and pharmacology* (Eds. Machy P and Leserman L), pp. 33-97. John Libbey and Company Ltd., London, 1987.

41. Weinstein JN, Fujimori K, Antibody-Mediated Drug Delivery. In: *Protein design and the development of new therapeutics and vaccines* (Eds. Hook JB and Poste G), pp. 359-370. Plenum Press, New York, 1990.

42. Ishihara H, Hara T, Aramaki Y, Tsuchiya S and Hosoi K, Reparation of Asialofetuin-Labeled Liposomes with Encapsulated Human Interferon-Gamma and Their Uptake by Isolated Rat Hepatocytes. *Pharm Res* 7: 542-546, 1990.

43. Yamashita C, Sone S, Ogura T and Kiwada H, Potential Value of Cetylmannoside-Modified Liposomes As Carriers of Macrophage Activators to Human Blood Monocytes. *Jpn J Cancer Res 82:* 569-576, 1991.

44. Spanjer HH and Scherphof GL, Targeting of lactosylceramide-containing liposomes to hepatocytes in vivo. *Biochim Bwphys Acta* 734: 40-47, 1983.

45. Bijsterbosch MK and Van Berkel TJC, Uptake of Lactosylated Low-Density Lipoprotein by Galactose-Specific Receptors in Rat Liver. *Biochem J* 270: 233-239, 1990.

46. Bijsterbosch MK, Ziere GJ and Van Berkel TJC, Lactosylated Low Density Lipoprotein: A Potential Carrier for the Site-Specific Delivery of Drugs to Kupffer Cells. *Mol Pharmacol* 36: 484-489, 1989.

47. Haensler J and Schuber F, Influence of the Galactosyl Ligand Structure on the Interaction of Galactosylated Liposomes with Mouse Peritoneal Macrophages. *Glycocon* J 8: 116-124, 1991.

48. Ishii Y, Aramaki Y, Hara T, Tsuchiya S and Fuwa T, Preparation of EGF labeled liposomes and their uptake by hepatocytes. *Biochem Biophys Res Commun 160:* 732-736, 1989.

49. Attie AD, Pittman RC and Steinberg D, Metabolism of native and of lactosylated human

low density lipoprotein, evidence for two pathways for catabolism of exogenous proteins in rat hepatocytes. *Proc Natl Acad Sci USA 77: 5923-5927,* 1980.

50. Tomlinson E, Theory and practice of site specific drug delivery. *Adv Drug Deliv Rev* 1: 87-198, 1987.
51. Suzuki H, Zelphati O, Hildebrand G and Leserman L, CD4 and CD7 Molecules As Targets for Drug Delivery from Antibody Bearing Liposomes. *Exp Cell Res* 193: 112-119, 1991.
52a. Juliano RL, Factors affecting the clearance kinetics and tissue distribution of liposomes, microspheres *and* emulsions. *Adv Drug Deliv Rev 2:* 31-54, 1988.
52b. Maruyama K, Mori A, Kennel SJ, Waalkes MVB, Scherphof GL and Huang L, Drug Delivery by Organ-Specific Immunoliposomes. In: *Polymeric Drug and Drug Delivery Systems no. 469* (Eds. Dunn RL and Ottenbrite RM), pp.275-284, 1991.
53. Ho RJY, Rouse BT and Huang L, Target-sensitive immunoliposomes as an efficient *drug* carrier for antiviral activity. J *Bid Chem 262:* 13973-13978, 1987.
54. Ho RJY, Rouse BT and Huang L, Interactions of target-sensitive immunoliposomes with Herpes Simplex Virus. The foundation of a sensitive immunoliposome assay for the virus. I *Biol Chem 262:* 13979-13984, 1987.
55. Van Berkel TJC, Kruyt JK and Kempen HJM, Specific targeting of high density lipoproteins to liver hepatocytes by incorporation of a tris-galactoside terminated cholesterol derivative. J Bid *Chem* 260: 12203-12207, 1985.
56. *Duncan* R, Selective endocytosis of macromolecular drug carriers. In: *Controlled Drug Delivery: Fundamentals and Applications* (Eds. Robinson JR and Lee VHL), pp. 581-621. Marcel Dekker, INC., New York, Basel, 1987.
57. Duncan R, Lloyd JB, Biological evaluation of soluble synthetic polymers as *drug* carriers. In: *Recent Advances in Drug Delivery Systems* (Eds. *Anderson* JM and Kim SW), pp. 9-22. Plenum Press, New York and London, 1984.
58. Duncan R, Kopecek J, Lloyd JB, Biological evaluation of soluble macromolecules as bioreversible *drug* carriers. In: *Bioreversible carriers in drug design. Theory and application (Ed.* Roche EB), pp. 196-213. Pergamon Press, New York, 1989.
59. Midoux P, Negre E, Roche AC, Mayer R, Monsigny M, Balzarini J, De Clercq E, Mayer E, Ghaffar A and Gangemi JD, Drug Targeting : Anti-HSV-1 Activity of Mannosylated Polymer-Bound 9-(2-Phosphonylmethoxyethyl)Adenine. *Biochem Biophys Res Commun 167:* 1044-1049, 1990.
60. Couvreur P, Polyalkylcyanoacrylates as colloidal *drug* carriers. *Crit Rev Ther Drug Carrier Syst 5:* 1-20, 1988.
61. Speiser PP, Nanoparticles and Liposomes: A State of the Art. *Methods Find Exp Clin Pharmacol* 13: 337-342, 1991.
62. Friend DR and Pangburn S, Site specific drug delivery, *Med Res Rev 7:* 53-106, 1987.
63. Codde JP, Burton MA, Kelleher DK, Archer SG and Gray BN, Reduced Toxicity of Adriamycin by Incorporation into Ion Exchange Microspheres: A Therapeutic Study Using a Rat Liver Tumour Model. *Anticancer Res* 10: 1715-1718, 1990.
64. Nicolau C and Cudd A, Liposomes as carriers of DNA. *Crit Rev Ther Drug Carrier Syst 6:* 239-271, 1989.
65. Roche AC, Midoux P, Pimpaneau V, Nègre E, Mayer R and Monsigny M, Endocytosis Mediated by Monocyte and Macrophage Membrane Lectins - Application to Antiviral Drug Targeting. *Res Virol* 141: 243-249, 1990.
66. Jansen RW, Molema G, Ching TL, Oosting R, Harms G, Moolenaar F, Hardonk MJ and Meijer DKF, Hepatic endocytosis of various types of mannose-terminated albumins. What is important, sugar recognition, net charge or the combination of these features. I *Bid Chem* 266: 3343-3348, 1991.
67. Shen WC and Ryser JP, Cis-aconityl spacer between daunomycin and macromolecular carriers: A model of pH-sensitive linkage releasing drug from a lysosomotropic conjugate. *Biochem Biophys Res Commun* 102: 1048-1054, 1981.
68. Stoorvogel W, Geuze HJ, Griffith JM, Schwartz AL and Strous GJ, Relation between intracellular pathways of the receptors for Transferrin, Asialoglycoprotein and

mannose-6-phosphate in human hepatoma cells. J Cell Bid 108: 2137-2148,1989.
69. Sugiyama Y and Hanano M, Receptor-mediated transport of peptide hormones and its importance in the overall hormone disposition in the body. *Pharm Res* 6: 192-202,1989.
70. Gabius HJ, Schroter C, Gabius S, Brinck U and Tietze LF, Binding of T-antigen-bearing neoglycoprotein and peanut agglutinin to cultured tumor cells and breast carcinomas. J *Histochem Cytochem* 38: 1625-1631, 1990.
71. Keegan-Rogers V and Wu GY, Targeted Protection of Hepatocytes from Galactosamine Toxicity In Vivo. *Cancer Chemother Pharmacol 26:* 93-96, 1990.
72. Wilson G, Gene Therapy: Rationale *and* Realisation. In: *Site Specific Drug Delivery* (Eds. Tomlinson E and Davis SS), pp. 149-164. John Wiley, Chichester, 1986.
73. Trouet A, Masquelier M, Baurain R and Deprez-De Campeneere D, A covalent linkage between daunorubicin and proteins that is stable in serum and reversible by lysosomal hydrolysis as required far a lysosomotropic drug–carrier conjugate: in vitro and in vivo studies. *Proc Natl Acad Sci USA* 79: 626-629,1982.
74. Franssen EJF, Van Amsterdam RGM, Visser J, Moolenaar F, De Zeeuw D and Meijer DKF, Low molecular weight proteins as carriers for renal drug targeting: Naproxen-Lysozyme. *Pharm Res* 8: 1223-1230, 1991.
75. Vitetta ES and Uhr JW, Immunotoxins. *Annu Rev Immund 3:* 197-212,1985.
76. Hwang KM, Foon KA, Cheung PH, Pearson JW and Oldham RK, Selective antitumor effect on L10 hepatocarcinoma cells of a potent immunoconjugate composed of the A chain of Abrin and a Monoclonal Antibody to a hepatoma-associated antigen. *Cancer Res* 44: 4578-4586,1984.
77. Olsnes S, Sandvig K, Petersen OW and Van Deurs B, Immunotoxins - Entry into Cells and Mechanisms of Action. *Immunol Today* 10: 291-2%. 1989.
78. Shea CR, Chen N, Wimberly J and Hasan T, Rhodamine Dyes As Potential Agents for Photochemotherapy of Cancer in Human Bladder Carcinoma *Cells. Cancer Res 49:* 3961-3965, 1989.
79. Ghetie MA, Uhr JW and Vitetta ES, Covalent Binding of Human alpha2-Macroglobulin to Deglycosylated Ricin A Chain and Its Immunotoxins. *Cancer Res* 51: 1482-1487, 1991.
80. Ghose T, Blair AH, Vaugham K, Kulkarni P, Antibody-directed *drug* targeting in cancer therapy. In: *Targeted Drugs* (Ed. Goldberg EP), pp. 1-22. John Wiley and Sons, New York, 1982.
81. Sehon AH, Immunological strategy for arresting the development of AIDS in clinically healthy, asymptomatic, HIV-infected individuals. In: *NATO ASI: Targeting of drugs: the challenge of peptides and proteins* (Eds. Gregoriadis G, Florence AT and Poste G), Cape Sounion Beech meeting 1991, abstract, 1991.
82. Vitetta ES, Immunotoxins: New Therapeutic Reagents for Autoimmunity, Cancer, and AIDS. J *Clin Immund 10:* S15-S18, 1990.
83. Vallera DA, Carroll SF, Snover DC, Carlson GJ and Blazar BR, Toxicity and Efficacy of Anti T-Cell Ricin Toxin A Chain Immunotoxins in a Murine Model of Established Graft-Versus-Host Disease Induced Across the Major Histocompatibility Barrier. *Blood* 77: 182-194, 1991.
84. Blakey DC, Thorpe PE, Prevention of carbohydrate-mediated clearance of ricin-containing immunotoxins by the liver. In: *Immunotoxins* (Ed. Frankel *AE),* pp. 457-473. Kluwer Academic Publishers, Boston/Dordrecht/Lancaster, 1988.
85. Zarling JM, Moran PA, Haffar O, Sias J, Richman DD, Spina CA, Myers DE, Kuebelbeck V, Ledbetter JA and Uckun FM, Inhibition of HIV Replication by Pokeweed Antiviral Protein Targeted to Cd4+ *Cells* by Monoclonal Antibodies. *Nature* 347: 92-95, 1990.
86. Kim Y-W, Fung MSC, Sun NC, Sun CRY, Chang NT and Chang TW, Immunoconjugates That Neutralize HIV Virions Kill T Cells Infected with Diverse Strains of HIV-1. J *Immunol* 144: 1257-1262,1990.
87. Uckun FM, Myers DE, Ledbetter JA, Wee SL and Vallera DA, Cell-Type Specific Cytotoxicity of Anti-CW and Anti-CD8 Ricin Immunotoxins Against Human Alloreactive T-Cell Clones. *Blood* 74: 2445-2454, 1989.
88. Till MA, Zolla-Pazner S, Gorny MK, Patton JS, Uhr JW and Vitetta ES, Human

Immunodeficiency virus-infected T cells and monocytes are killed by monoclonal human anti-gp41 antibodies coupled to ricin A chain. *Proc Natl Acad Sci USA 86:* 1987-1991, 1989.

89. Matsushita S, Koito A, Maeda Y, Hattori T and Takatsuki K, Selective Killing of HIV-Infected Cells by Anti-Gp120 Immunotoxins. *AIDS Res* Hum *Retroviruses 6:* 193-203, 1990.
90. Pincus SH, Wehrly K and Chesebro B, Treatment of HIV tissue culture infection with monoclonal antibody-ricin A chain conjugates. J *Immunol* 142: 3070-3075,1989.
91. Barbieri L, Bolognesi A, Dinota A, Lappi DA, Soria M, Tazzari PL and Stirpe F, Selective Killing of Cd4+ and Cd8+ Cells with Immunotoxins Containing Saporin. *Scand J Immunol* 30: 369-372, 1989.
92. Senter PD, Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy. *FASEB J 4:* 188-193, 1990.
93. Bagshawe KD, Springer CJ, Searle F, Antoniw P, Sharma SK, Melton RG and Sherwood RF, A cytotoxic agent can be generated selectively at cancer sites. *Br J Cancer 58:* 700-703,1988.
94. Senter PD, Saulnier MG, Schreiber GJ, Hirschberg DL, Brown JP, Hellström I and Hellström KE, Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate. Proc *Natl Acad Sci USA 85:* 4842-4846, 1988.
95. Nelson H, Targeted cellular immunotherapy with bifunctional antibodies. *Cancer Cells* 3: 163-172, 1991.
96. Berg J, Lōtscher E, Steimer KS, Capon DJ, Baenziger J, Jäck H-M and Wabl M, Bispecific Antibodies That Mediate Killing of Cells Infected with Human Immunodeficiency Virus of Any Strain. Proc *Natl Acad Sci USA 88:* 4723-4727, 1991.
97. Till MA, Ghetie V, Gregory T, Patzer EJ, Porter JP, Uhr JW, Capon DJ and Vitetta ES, HIV-infected cells are killed by rCD4-ricin A chain. *Science* 242: 1166-1168, 1988.
98. Chaudhary VK, Mizukami T, Fuerst TR, Fitzgerald DJ, Moss B, Pastan I and Berger EA, Selective killing of HIV-infected cells by recombinant human CD4-Pseudomonas exotoxin hybrid protein. *Nature* 335: 369-372, 1988.
99. Ashorn P, Moss B, Weinstein JN, Chaudhary VK, Fitzgerald DJ, Pastan I and Berger EA, Elimination of Infectious Human Immunodeficiency Virus from Human T-Cell Cultures by Synergistic Action of CD4-Pseudomonas Exotoxin and Reverse Transcriptase Inhibitors. Proc *Natl Acad Sci USA 87:* 8889-8893, 1990.
100. Lloyd JB, Soluble polymers as targetable drug carriers. In: *Drug delivery systems: Fundamentals and Techniques* (Eds. Johnson P and Lloyd-Jones JG), pp. 95-105. Ellis Horwood, 1987.
101. Cartlidge SA, Duncan R, Lloyd JB, Kopeckova-Rejmanova P and Kopecek J, Soluble crosslinked N-(2-hydroxypropyl)methacrylamide copolymers as potential drug camers. 3. Targeting by incorporation of galactosamine residues. Effect of route of administration. J *Contr Rel 4:* 265-278, 1987.
102. Kopecek J, Rejmanova P, Duncan R and Lloyd JB, Controlled release of drug model from N-(2-hydroxypropyl)methacrylamide copolymers. *Ann N Y Acad Sci 446:* 93-104, 1984.
103. Jaroszewski JW and Cohen JS, Cellular uptake of antisense oligodeoxynucleotides. *Adv Drug Deliv Rev 6:* 235-250, 1991.
104. Agrawal S and Sarin PS, Antisense oligonucleotides: gene regulation and chemotherapy of AIDS. *Adv Drug Deliv Rev 6:* 251-270, 1991.
105. Shoji Y, Akhtar S, Periasami A, Herman B and Juliano RL, Mechanism of cellular uptake of modified oligodeoxynucleotides containing methylphosphonate linkages. *Nucleic Acids Res 19:* 5543-5550, 1991.
106. Kabanov AV, Vinogradov SV, Ovcharenko AV, Krivonos AV, Melik-Nubarov NS, Kiselev VI and Severin ES, A New Class of Antivirals: Antisense Oligonucleotides Combined with a Hydrophobic Substituent Effectively Inhibit Influenza Virus Reproduction and Synthesis of Virus-Specific Proteins in MDCK Cells. *FEBS Lett* 259: 327-330, 1990.
107. Degols G, Leonetti J-P, Gagnor C, Lemaitre M and Lebleu B, Antiviral Activity and Possible Mechanisms of Action of Oligonucleotides-Poly(L-Lysine) Conjugates Targeted to

Vesicular Stomatitis Virus mRNA and Genomic RNA. *Nucleic Acids Res* 17: 9341-9350, 1989.

108. Stevenson M and Iversen PL, Inhibition of Human Immunodeficiency Virus Type 1-Mediated Cytopathic Effects by Poly(L-Lysine)-Conjugated Synthetic Antisense Oligodeoxyribonucleotides. J Gen *Virol 70:* 2673-2682,1989.
109. Leonetti J-P, Degols G and Lebleu B, Biological activity of oligonucleotide-poly(l-lysine) conjugates: mechanism of cell uptake. *Bioconjugate Chemistry* 2: 149-153,1990.
110. Zenke M, Steinlein P, Wagner E, Cotten M, Beug H and Birnstiel ML, Receptor-Mediated Endocytosis of Transferrin-Polycation Conjugates: An Efficient Way to Introduce DNA into Hematopoietic Cells. *Proc Natl Acad Sci USA 87:* 3655-3659,1990.
111. Forgac M, Receptor-mediated endocytosis. In: *The Liver: Biology and Pathobiology* (Eds. Arias IM, Jakoby WB, Popper H, Schachter D and Shafritz DA), pp. 207-225. Raven Press, New York, 1988.
112. Steer CJ, Ashwell G, Hepatic membrane receptors for glycoproteins. In: *Progress in Liver Diseases. Vd. VIII* (Eds. Popper H and Schaffner F), pp. 99-123. Grune and Stratton, New York, 1986.
113. Wu GY, Targeting in diagnosis and therapy. In: The *Liver: Biology and Pathobiology* (Eds. Arias IM, Jakoby WB, Popper H, Schachter D and Shafritz DA), pp. 1303-1313. Raven Press, New York, 1988.
114. Ashwell G and Morell AG, The role of surface carbohydrates in the hepatic recognition and transport of glycoproteins. *Adv Enzymol Relat Areas Mol Bid* 41: 99-128,1974.
115. Sasaki AW, Williams SK, Jain M and Wagner RC, Mechanism of sucrose uptake by isolated rat hepatocytes. J *Cell Physiol* 133: 175-180, 1987.
116. Hoppe CA and Lee YC, The binding and processing of mannose-BSA derivatives by rabbit alveolar macrophages. Effect of the sugar density. J *Bid Chem 258:* 14193-14199,1983.
117. Summerfield JA, Vergalla J and Jones A, Modulation of a glycoprotein recognition system on rat hepatic endothelial cells by glucose and diabetes mellitus. J *Clin Invest 69:* 1337-1347, 1982.
118. Schlesinger PH, Rodman JS, Frey M, Lang S and Stahl P, Clearance of lysosomal hydrolases following intravenous infusion. The role of liver in the clearance of B-Glucuronidase and N-Acetyl-B-D-Glucosaminidase. *Arch Biochem Biophys* 177: 606-614, 1976.
119. Bezouska K, Taborsky O, Kubrycht J, Pospisil M and Kocourek J, Carbohydrate-structure-dependent recognition of desialylated serum glycoproteins in the liver and leucocytes. Two complementary systems. *Biochem* J 227: 345-354, 1985.
120. Bodmer JL and Dean RT, Drug and Enzyme Targeting. *Methods Enzymol* 112: 2%-306, 1988.
121. Stowell CP and Lee YC, Neoglycoproteins: the preparation and application of synthetic glycoproteins. *Adv Carbohydr Chem Biochem 37:* 225-281,1980.
122. Fiume L, Bassi B, Busi C, Mattioli A and Spinoza G, Drug targeting in antiviral chemotherapy. A chemically stable conjugate of 9-beta-D-arabinofuranosyl-adenine 5' monophosphate with lactosaminated albumin accomplishes a selective delivery of the drug to liver cells. *Biochem Pharmacol* 35: 967-972, 1986.
123. Fiume L, Busi C, Mattioli A, Balboni PG, Barbanti-Brodano G, Wieland T, Hepatocyte targeting of antiviral drugs coupled to galactosyl-terminated glycoproteins. In: *Targeting of Drugs* (Eds. Gregoriadis G, Senior J and Trouet A), pp. 1-17. Plenum Press, New York, 1982.
124. Fiume L, Bonino F, Mattioli A, Chiaberge E, Cerenzia MRT, Busi C, Brunetto MR and Verme G, Inhibition of Hepatitis B Virus Replication by ara-AMP conjugated with lactosaminated serum albumin. *Lancet* July 2: 13-15, 1988.
125. Ponzetto A, Fiume L, Forzani B, Song SY, Busi C, Mattioli A, Spinelli C, Marinelli M, Smedile A, Chiaberge E, Bonino F, Gervasi G,B., Rapicetta M and Verme G, Adenine Arabinoside Monophosphate and Acyclovir Monophosphate coupled to Lactosaminated Albumin reduce Woodchuck Hepatitis Virus viremia at lower doses than do the unconjugated *drugs. Hepatology* 14: 16-24,1991.

Bacha P, Williams DP, Waters C, Williams JM, Murphy JR and Strom TB, Interleukin 2 receptor-targeted cytotoxicity. Interleukin 2 receptor-mediated action of a diphteria toxin-related Interleukin 2 fusion *protein. J Exp Med* 167: 612-622, 1988.

127. Waters CA, Schimke PA, Snider CE, Itoh K, Smith KA, Nichols JC, Strom TB and Murphy JR, Interleukin 2 Receptor-Targeted Cytotoxicity. Receptor Binding Requirements for Entry of a Diphtheria Toxin-Related Interleukin 2 Fusion Protein into Cells. *Eur J Immunol* 20: 785-791,1990.
128. Kiyokawa T, Williams DP, Snider CE, Strom TB and Murphy JR, Protein Engineering of Diphtheria-Toxin-Related Interleukin-2 Fusion Toxins to Increase Cytotoxic Potency for High-Affinity IL-2-Receptor-Bearing Target Cells. *Protein Eng 4:* 463-468, 1991.
129. Schwartz BA and Gray GR, Proteins containing reductively aminated disaccharides: synthesis and characterization. Arch *Biochem Biophys* 181: 542-549, 1977.
130. Kataoka H and Tavassoli M, Identification of lectin like substances recognizing galactosyl d u e s of glycoconjugates in the plasma membrane of marrow sinus endothelium. *Blood* 65: 1165-1171, 1985.
131. Jansen RW, Molema G, Harms G, Kruijt JK, Van Berkel TJC, Hardonk MJ and Meijer DKF, Formaldehyde treated albumin contains monomeric and polymeric forms that are differently cleared by endothelial and Kupffer cells of the liver: evidence for scavenger receptor heterogeneity. *Biochem Biophys Res Commun* 180: 23-32,1991.
132. Van der Sluijs P, Bootsma HP, Postema B, Moolenaar F and Meijer DKF, Drug targeting to the liver with lactosylated albumins. Does the glycoprotein target the *drug,* or is the drug targeting the glycoprotein? *Hepatology* 6: 723-728, 1986.
133. Fiume L, Mattioli A, Busi C, Balboni PG, Barbanti-Brodano G, De Vries J, Altmann R and Wieland T, Selective inhibition of Ectromelia virus DNA synthesis in hepatocytes by adenine-9-beta-D-arabinofuranoside (ara-A) and adenine-9-arabinofuranoside monophosphate (ara-AMP) conjugated to asialofetuin. *FEBS Lett* 116: 185-188, 1980.
134. Baum RM, AIDS. The molecular biology. *Chem Eng* N november 23: 14-26, 1987.
135. Baum RM, Aids researchers make inroads in understanding a complex virus. *Chem Eng N* december 1: 7-12, 1986.
136. Kurth R, Does HIV Cause AIDS - An Updated Response to Duesberg's Theories. *Intervirology* 31: 301-314, 1990.
137. Mccune JM, HIV-1: The Infective Process In Vivo. *Cell* 64: 351-363,1991.
138. Mann J, Global AIDS into the 1990s. *World Health* october: 6-7, 1989.
139. Sato PA, Chin J, Mann JM, Acquired Immunodefiency Syndrome (AIDS): An International Perspective. In: The *human retroviruses* pp. 213-224. Academic Press, 1991.
140. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Axler-Blin C, Brun-Vezinet F, Rouzioux C, Rozenbaum W and Montagnier L, Isolation of a T-lymphotropic retrovirus from a patient at risk for the Acquired Immune Deficiency Syndrome (AIDS). *Science* 220: 868-871,1983.
141. Popovic M, Sarngadharan MG, Read E and G d o RC, Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224: 497-500, 1984.
142. Lifson J, Coutre S, Huang E and Engleman E, Role of envelope glycoprotein carbohydrate in HIV infectivity and virus induced cell fusion. *J Exp Med* 164: 2101-2106,1986.
143. Mcdougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A and Nicholson JKA, Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110 K viral protein and *the* T4 molecule. *Science* 231: 382-385, 1986.
144. Sattentau QJ and Weiss RA, The CD4 antigen: Physiological ligand *and* HIV receptor. *Cell* 52: 631-633, 1988.
145. Klatzmann D, Barré-Sinoussi FB, Nugeyre MT, Dauguet C, Vilmer E, Griscelli E, Brun-Vezinet F, Rouzioux C, Gluckman JC, Chermann JC and Montagnier L, Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* 225: 59-63, 1984.
146. Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC and Popovic M, The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 233: 215-219,1986.

147. Ho DD, Rota TR and Hirsch MS, Infection of monocyte/macrophages by Human T Lymphotropic Virus Type III. J *Clin* Invest *77:* 1712-1715, 1986.
148. Perno CF, Yarchoan R, Cooney DA, Hartman NR, Webb DSA, Hao Z, Mitsuya H, Johns DG and Broder S, Replication of human immunodeficiency virus in monocytes. Granulocyte/Macrophage Colony-stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3'-azido-2'3'-dideoxythymidine (AZT) and other dideoxynucleoside congeners of thymidine. J *Exp Med* 169: 933-951, 1989.
149. Gendelman HE, Orenstein JM, Baca LM, Weiser B, Burger H, Kalter DC and Meltzer MS, The Macrophage in the Persistence and Pathogenesis of HIV Infection. *AIDS* 3: 475-495, 1989.
150. Scoazec JY and Feldmann G, Both Macrophages and Endothelial Cells of the Human Hepatic Sinusoid Express the CD4 Molecule, a Receptor for the Human Immunodeficiency Virus. *Hepatology* 12: 505-510,1990.
151. Steffan AM, Gendrault JL, Schweitzer C, Schmitt MP, Royer C, Aubertin AM, Jaeck D, Lafon ME, Kirn A, Multiplication of Human Immunodeficiency Virus Type-I (HIV-1) in Cultured Human Endothelial Liver Cells. In: *Cells of the Hepatic Sinusoid* (Eds. Wisse E, Knook DL and McCuskey RS), pp. 417-422. Kupffer Cell Foundation, Leiden, 1991.
152. Schmitt MP, Steffan AM, Gendrault JL, Jaeck D, Royer C, Schweitzer C, Beyer C, Schmitt C, Aubertin AM and Kirn A, Multiplication of Human Immunodeficiency Virus in Primary Cultures of Human Kupffer Cells - Possible Role of Liver Macrophage Infection in the Physiopathology of AIDS. *Res* Vird 141: 143-152,1990.
153. Gendrault JL, Steffan AM, Schmitt MP, Jaeck D, Aubertin AM and Kirn A, Interaction of Cultured Human Kupffer Cells with HIV-Infected CEM Cells: An Electron Microscopic Study. *Pathobiology* 59: 223-226,1991.
154. Lucey DR, Dorsky DI, Nicholson-Weller A and Weller PF, Human eosinophils express CD4 protein and bind human immunodeficiency virus gp120. J *Exp Med* 169: 327-332, 1989.
155. Schmitt D, Dezutter-Dambuyant C, Hanau D, Kolbe HVJ, Kieny MP, Cazenave JP and Thivolet J, In Vitro Binding and Internalization of HIV Envelope Glycoproteins by Human Epidermal Langerhans Cells Does Not Require the CD4-Gp120-Binding Site. *Res Vird* 141: 209-215, 1990.
156. Wood GS, Warner NL and Warnke RA, Anti-Leu-3/T4 antibodies react with cells of monocyte/macrophages and langerhans lineage. J *Immunol* 131: 212-216, 1983.
157. Mann DL, Read-Connole E, Arthur LO, Robey WG, Wernet P, Schneider EM, Blattner WA and Popovic M, HLA-DR is involved in the HIV-1 biding site on cells expressing MHC class II antigens. J *Immunol* 141: 1131-1136,1988.
158. Diamond DC, Finberg R, Chaudhuri S, Sleckman BP and Burakoff SJ, Human Immunodeficiency Virus Infection Is Efficiently Mediated by a Glycolipid-Anchored Form of CD4. *Proc Natl* Acad *Sci USA 87:* 5001-5005, 1990.
159. Bhat S, Spitalnik SL, Gonzalez-Scarano F and Silberberg DH, Galactosyl Ceramide or a Derivative Is an Essential Component of the Neural Receptor for Human Immunodeficiency Virus Type-1 Envelope Glycoprotein gp120. Proc *Natl Acad Sci USA* 88: 7131-7134,1991.
160. Lusso P, Lori F and Gallo RC, CD4-independent infection by human immunodeficiency virus type 1 after phenotypic mixing with human T-cell leukemia viruses. J *Vird* 64: 6341-6344, 1990.
161. Tateno M, Gonzalez-Scarano F and Levy JA, Human Immunodeficiency Virus Can Infect CD4-Negative Human Fibroblastoid Cells. Proc *Natl Acad Sci USA 86:* 4287-4290, 1989.
162. Filice G, Cereda PM, Orsolini P, Soldini L, Razzini E, Campisi D and Gulminetti R, Endocytosis Constitute the Infectious Route of HIV-1 Entry in Human and Rabbit Monocytes Lacking the CD4 Receptor. *Microbiologica* 14: 77-93, *1991.*
163. Gruters RA, Neefjes JJ, Tersmette M, De Goede REY, Tulp A, Huisman HG, Miedema F and Ploegh HL, Interference with HIV-induced syncytium formation and viral infectivity by inhibitors of trimming glucosidase. *Nature 330:* 74-77, 1987.
164. Müller WEG, Renneisen K, Kreuter MH, Schroder HC and Winkler 1, The D-mannose

specific lectin from Gerardia Savaglia blocks binding of HIV-1 to H9 cells and human lymphocytes in vitro. J *Acquir Immune Defic Syndr* 1: 453-458, 1988.

165. Matthews TJ, Weinhold KJ, Lyerly HK, Langlois AJ, Wigzell H and Bolognesi DP, Interaction between the human T-cell lymphotropic virus type IIIB envelop glycoprotein gp120 and the surface antigen CD4: Role of carbohydrate in binding and cell fusion. *Proc Natl Acad Sci USA* 84: 5424-5428, 1987.
166. Ezekowitz RAB, Kuhlman M, Groopman JE and Byrn RA, A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. J Exp *Med* 169: 185-1%, 1989.
167. Robinson WE, Montefiori DC and Mitchell WM, Evidence that mannosyl residues are involved in Human Immunodeficiency Virus type 1 (HIV-1) pathogenesis. *AIDS Res* Hum *Retroviruses 3:* 265-282,1987.
168. Fenouillet E, Clerget-Raslain B, Gluckman JC, Guetard D, Montagnier L and Bahraoui E, Role of N-linked glycans in the interaction between the envelope glycoprotein of Human Immunodeficiency Virus and its CD4 cellular receptor. J Exp *Med* 169: 807-822,1989.
169. Dirckx L, Lindemann D, Ette R, Manzoni C, Moritz D and Mous J, Mutation of Conserved N-Glycosylation Sites Around the CW-Binding Site of Human Immunodeficiency Virus *Type* 1 GP120 Affects Viral Infectivity. *Virus Res* 18: 9-20, 1990.
170. Fenouillet E, Gluckman JC and Bahraoui E, Role of N-Linked Glycans of Envelope Glycoproteins in Infectivity of Human Immunodeficiency Virus Type-1. J *Vird* 64: 2841-2848, 1990.
171. Larkin M, Childs RA, Matthews TJ, Thiel S, Mizuochi T, Lawson AM, Savill JS, Haslett C, Diaz R and Feizi T, Oligosaccharide-Mediated Interactions of the Envelope Glycoprotein Gp120 of HIV-1 That Are Independent of CD4 Recognition. *AIDS* 3: 793-798, 1989.
172. Botarelli P, Houlden BA, Haigwood NL, Servis C, Montagna D and Abrignani S, N-Glycosylation of HIV-gp120 May Constrain Recognition by T Lymphocytes. J *Immund* 147: 3128-3132,1991.
173. Lifson JD, Feinberg MB, Reyes GR, Rabin L, Banapour B, Chakrabarti S, Moss B, Wong-Staal F, Steimer KS and Engleman EG, Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature* 3W: 725-728, 1986.
174. Stein BS, Gowda SD, Lifson JD, Penhallow RC, Bensch KG and Engleman EG, pH-independent HIV entry into CW-positive T-cells via virus envelope fusion to the plasma membrane. *Cell* 49: 659-668,1987.
175. Burny A, Bex F, Brasseur R, Chuah Lay Khim M, Delchambre M, Horth M and Verdin E, Human immunodeficiency virus cell entry: new insights into the fusion mechanism. J *Acquu Immune Defic Syndr* 1: 579-582,1988.
176. Pauza CD and Price TM, Human Immunodeficiency Virus infection of T cells and monocytes proceeds via receptor-mediated endocytosis. *J Cell Bid* 107: 959-968, 1988.
177. Goto T, Harada S, Yamamoto N and Nakai M, Entry of human immunodeficiency virus (HIV) into MT-2, human T cell leukemia virus carrier cell line. Arch Vird 102: 29-38, 1988.
178. De Clercq E, Chemotherapeutic approach of AIDS. In: *Verhandelingen van de koninklijke academie voor geneeskunde van Belgie, L nr. 2* pp. 166-217. 1988.
179. Johnston MI, Allaudeen HS and Sarver N, HIV proteinase as a target for drug action. *Trends Pharmacol Sci* 10: 305-307,1989.
180. Michael NL, Vahey M, Burke DS and Redfield RR, Viral DNA and mRNA Expression Correlate with the Stage of Human Immunodeficiency Virus (HIV) *Type* 1 Infection in Humans: Evidence for Viral Replication in All Stages of HIV Disease. J Vird 66: 310-316, 1992.
181. Somasundaran M and Robinson HL, A major mechanism of human immunodeficiency virus-induced cell killing does not involve cell fusion. J *Vird* 61: 3114-3119, 1987.
182. Petteway SR,Jr., Lambert DM and Metcalf BW, The Chronically Infected Cell As a Target for the Treatment of HIV Infection and AIDS. *Trends Pharmacol Sci* 12: 28-33,1991.
183. Pauza CD, HIV persistence in monocytes leads to pathogenesis and AIDS. *Cell Immund*

112: 414-424,1938.

184. Jouault T, **Chapuis F, Bahraoui** E and **Gluckman** J-C, Infection of **Monocytic** Cells by **HIV1: Combined** Role *of* **FcR** and **CD4.** *Res Virol* 142: 183-188,1991.

185. **Gartner** S and **Popovic** M, **Macrophage** tropism *of* **HIV-1.** *AIDS Res Hum Retroviruses 6:* 1017-1021, **1990.**

186. Cloyd MW and Lynn WS, Perturbation of **Host-Cell** Membrane Is a Primary **Mechanism** cl HIV **Cytopathology.** *Virology* 181: 500-511,1991.

187. **Sodroski** J, **Goh** WC, **Rosen** C, **Kampbell K** and Haseltine **WA, Role** *of* the **HTLV-II/LAV** envelope in **syncytium formation** *and* **cytopathicity.** *Nature* 322: 470474, 1986.

188. **Hofmann** B, Nishanian P, Baldwin **RL, Insixiengmay** P, **Nel** A and **Fahey** JL, HIV Inhibits the Early Steps of Lymphocyte Activation, Including Initiation of Inositol Phospholipid **Metabolism.** J *Immunol* **145:** 3699-3705,1990.

189. **Cefai** D, Debre P, **Kaczorek** M, **Idziorek** T, Autran B and **Bismuth** G, Human **Immunodeficiency Virus-1 Glycoproteins gp120 and gp160 Specifically Inhibit** the CD3/T Cell-Antigen Receptor **Phosphoinositide Transduction** Pathway. J *Clin Invest* **86**: 2117-2124,1990.

190. **Agy MB, Wambach** M, Foy K and Katze MG, Expression of Cellular **Genes** in CD4 Positive Lymphoid Cells Infected by **the** Human Immunodeficiency Virus, HIV-1 Evidence for a Host **Protein Synthesis** Shut-Off **Induced** by Cellular **Messenger** RNA **Degradation.** *Virology* 177: 251-258, **1990.**

191. **Oyaizu** N, **Chirmule** N, **Kalyanaraman** VS, Hall **WW,** Good **RA** and **Pahwa** S, **Human Immunodeficiency** Virus Type-1 Envelope **Glycoprotein Gp120 Produces Immune Defects** in Cd4+ Lymphocytes-T by Inhibiting Interleukin-2 Messenger **RNA.** *Proc Natl Acad Sci USA 87:* 2379-2383,1990.

192. **Gurley** RJ, **Ikeuchi** K, **Byrn** RA, **Anderson K** and Groopman JE, **CD4+** lymphocyte function with early human immunodeficiency *virus* infection. *Proc Natl Acad Sci USA* **86:** 1993-1997, 1989.

193. **Nokta** M and **Pollard** R, Human **Immunodeficiency** Virus Infection: Association with Altered **Intracellular Levels** of **cAMP and cGMP** in **MT-4 Cells.** *Virology* 181: 211-217, 1991.

194. **Aguilar** JJ, **Anel A, Torres JM, Semmel M** and **Uriel** J, Changes in Lipid Composition of Human Peripheral Blood Lymphocytes Infected by **HIV.** *AIDS Res Hum Retroviruses 7:* **761-765,** 1991.

195. **Andreesen** R, **Brugger** W, **Kunze** R, **Stille** W and **Von Briesen** H, Defective **Monocyte** to **Macrophage Maturation** in Human Immunodeficiency Virus Infection. *Res Virol* 141: **217-224, 1990.**

196. Balleari E, **Timitilli** S, Puppo F, **Gaffuri** L, **Musselli** C, Rizzo F, **Indiveri** F and **Ghio** R, Impaired In **Vitro** Growth of Peripheral Blood **Hematopoietic** Progenitor *Cells* in HIV-Infected Patients: **Evidence** of an Inhibitory Effect of **Autologous T-Lymphocytes.** *Ann Hematol* 63: **320-325, 1991.**

197. **Chalmers** AH, Hare C, **Woolley** G and **Frazer** IH, Lymphocyte **Ectoenzyme Activity** Compared in Healthy **Persons and** Patients **Seropositive** to or at High **Risk** of HIV Infection. *Immunol Cell* Bid *68:* 81-85,1990.

198. **Lafeuillade A, Alessi** MC, **Poizotmartin** I, **Boyerneumann** C, **Zandotti** C, **Quilichini** R, **Aubert** L, **Tamalet** C, **Juhanvague** 1 and **Gastaut JA, Endothelial** *Cell* **Dysfunction** in HIV Infection. J *Acquir Immune Defic Syndr 5:* 127-131,1992.

199. **Baba** M, **Nakajima** M, **Schols** D, **Pauwels** R, **Balzarini** J and **De Clercq** E, **Pentosan polysulfate,** a sulfated **oligosaccharide,** is a potent **and** selective anti-HIV agent in **vitro.** *Antiviral Res* 9: 335-343, **1988.**

200. **Baba** M, **Snoeck** R, **Pauwels** R and De **Clercq** E, Sulfated **polysaccharides** are potent and **selective** inhibitors of various **enveloped** viruses, including **herpes** simplex virus, **cytomegalovirus,** vesicular **stomatitis** virus and human **immunodeficiency** virus. *Antimicrob Agents Chemother* 32: 1742-1745, 1988.

201. De **Clercq** E, **Basic approaches** to **anti-retroviral** treatment. J *Acquir Immune Defic Syndr*

4: 207-218, 1991.

202. Balzarini J, Mitsuya H, De Clerq E and Broder S, **Aurintricarboxylic** acid and Evans blue represent two different classes of anionic compounds which selectively inhibit the **cytopathogenicity** of human **T-cell lymphotropic** virus **type III/lymphadenopathy associated** virus. ***Biochem Biophys Res Commun* 136:** 64-71,1986.

203. **Cushman** M, **Wang** P, **Chang** SH, Wild C, De Clerq E, **Schols** D, **Goldman** ME and **Bowen** JA, Preparation and Anti-HIV Activities **of Aurintricarboxylic** Acid Fractions and Analogues: **Direct** Correlation of Antiviral **Potency** with Molecular Weight. ***J Med Chem* 34: 329-337, 1991.**

204. Fisher RA, Bertonis **JM,** Meier W, Johnson VA, **Costopoulos** DS, **Liu** T, Tizard R, **Walker** BD, Hirsch **MS, Schooley** RT and **Flavell** RA, **HIV** infection is blocked in vitro by **recombinant soluble CD4.** *Nature* 331: 76-78, **1988.**

205. **Hussey** RE, **Richardson NE, Kowalski** M, Brown NR, **Chang** HC, **Siliciano** RF, **Dorfman** T, Walker B, Sodroski J and **Reinherz** EL, A **soluble CD4** protein selectively inhibits **HIV replication** *and* **syncytium** formation. *Nature* 331: 78-81,1988.

206. **Traunecker** A, **Lüke** W and Karjalainen K, Soluble **CD4** molecules **neutralize** human **immunodeficiency** virus type 1. *Nature* 331: 84-86, 1988.

207. Hart TK, Kirsh R, Ellens H, Sweet RW, Lambert DM, **Petteway SR,Jr., Leary** J and **Bugelski** PJ, **Binding** of Soluble **CD4** Proteins to Human **Immunodeficiency** Virus **Type** 1 and **Infected** Cells Induces **Release** of Envelope **Glycoprotein gp120.** ***Proc Natl Acad Sci USA 88:*** 2189-2193,1991.

208. **Balzarini J,** Schols D, **Neyts** J, Van **Damme** E, **Peumans** W and De **Clercq** E, ***α-(2-3)-***and ***α-*(1-6)-D-Mannose-Specific** Plant Lectins Are Markedly Inhibitory to **Human Immunodeficiency** Virus **and Cytomegalovirus Infections** In Vitro. ***Antimicrob Agents Chemother* 35:** 410-416, 1991.

209. **Jansen** RW, **Molema G,** Pauwels R, **Schols** D, De **Clercq** E **and Meijer** DKF, Potent in vitro **anti-HIV-1** activity of modified human serum albumins. ***Mol Pharmacol*** 39: 818-823, 1991.

210. **Yarchoan** R, Thomas RV, Allain ***JP,* McAtee** N, **Dubinsky** R, Mitsuya H, **Lawley** TJ, Safai B, Myers CE, **Perno CF, Klecker** RW, Wills RJ, Fischl MC, **McNeely** MC, **Pluda** JM, **Leuther** M, Collins JM and Broder S, Phase I **studies of 2',3'-dideoxycytidine** in severe human immunodeficiency virus infection as a single agent **and** alternating with **zidovudine** (AZT). ***Lancet* january** 16: **76-80, 1988.**

211. Mitsuya H, **Jarrett** RF, **Matsukura** M, **Veronese** FD, DeVico AL, **Sarngadharan** MG, Johns DG, **Reitz MS** and Broder S, **Long-term** inhibition of human **T-lymphotropic** virus type **III/lymphadenopathy-associated** virus (human **immunodeficiency** virus) DNA synthesis and RNA **expression** in T cells protected by **2',3'-dideoxynucleosides in** vitro. ***Proc Natl Acad Sci USA 84:*** 2033-2037, 1987.

212. **Cooley** TP, **Kunches** LM, **Saunders** CA, **Perkins** CJ, **Kelley** SL, **McLaren** C, **McCaffrey** RP and **Liebman** HA, Treatment of AIDS and AIDS-related **complex** with **2',3'-dideoxyinosine** given **once** daily. ***Rev Infect Dis*** 12: **S552-S560, 1990.**

213. **Yarchoan** R, **Mitsuya** H, Thomas RV, **Pluda** JM, **Hartman** NR, **Perno** CF, Marczyk KS, Allain JP, Johns DG **and** Broder S, In Vivo Activity Against **HIV and** Favorable Toxicity **Profile *of* 2',3'-Dideoxyinosine.** ***Science* 245:** 412-415,1989.

214. Fischl MA, Richman DD, **Grieco** MH, **Gottlieb MS, Volberding** PA, **Laskin** OL, Leedom JM, **Groopman** JE, **Mildvan** D, **Schooley** RT, Jackson GG, Durack DT, Phil D and **King** D, The efficacy of azidothymidine (AZT) in the **treatment of patients** with ***AIDS*** and AIDS-related wmplex. ***N Engl* J *Med*** 317: 185-191, 1987.

215. Mitsuya H, **Weinhold** KJ, Furman PA, **St.Clair** MH, Nusinoff-Lehrman S, **Gallo** RC, **Bolognesi** D, Barry DW and Broder S, **3'-azido-3'-deoxythymidine** (BW **A509U):** an antiviral agent that inhibits the infectivity **and cytopathic effect** of **human T-lymphotropic** virus type III/lymphadenopathy-associated virus in vitro. ***Proc Natl Acad Sci USA 82:*** 70%-7100,1985.

216. Pauwels R, **Andries** K, Desmyter J, **Schols** D, Kukla MJ, **Breslin** HJ, **Raeymaeckers** A, Van **Gelder** J, **Woestenborghs** R, **Heykants** J, Schellekens K, **Janssen MAC,** De Clerq E

and Janssen PAJ, Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. *Nature* 343: 470474, 1990.

217. White EL, Buckheit RW, Ross LJ, Germany JM, Andries K, Pauwels R, Janssen PAJ, Shannon WM and Chirigos MA, A TIBO Derivative, R82913, Is a Potent Inhibitor of HIV-1 Reverse Transcriptase with Heteropolymer Templates. *Antiviral Res* 16: 257-266, 1991.

218. Yarchoan R, Mitsuya H and Broder S, AIDS therapies. *Sci Am* october: 88-97, 1988.

219. Goodchild J, Agrawal S, Civeira MP, Sarin PS, Sun D and Zamecnik PC, inhibition of human imunodeficiency virus replication by antisense oligodeoxynucleotides. *Proc Natl Acad Sci USA* 85: 5507-5511, 1988.

220. Sarin PS, Agrawal S, Civeira MP, Goodchild J, Ikeuchi T and Zamecnik PC, Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. *Proc Natl Acad Sci USA 85:* 7448-7451, 1988.

221. Montefiori DC, Robinson WE,Jr. and Mitchell WM, Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1. *Proc Natl Acad Sci USA 85:* 9248-9252, 1988.

222. Blough HA, Pauwels R, De Clercq E, Cogniaux J, Sprecher-Goldberger S and Thiry L, Glycosylation inhibitors block the expression of LAV/HTLV-III (HIV) glycoproteins. *Biochem Biophys Res Commun 141:* 33-38,1986.

223. Walker BD, Kowalski M, Chun Goh W, Kozarsky K, Krieger M, Rosen C, Rohrschneider L, Haseltine W and Sodroski J, Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc Natl Acad Sci USA* 84: 8120-8124, 1987.

224. Meek TD, Lambert DM, Dreyer GB, Carr TJ, Tomaszek TA,Jr., Moore ML, Strickler JE, Debouck C, Hyland LJ, Matthews TJ, Metcalf BW and Petteway SR, Inhibition of HIV-1 Protease in Infected T-Lymphocytes by Synthetic Peptide Analogues. *Nature* 343: 90-92, 1990.

225. Huff JR, HIV Protease: A Novel Chemotherapeutic Target for AIDS. J *Med Chem 34:* 2305-2314, 1991.

226. Desjarlais RL, Seibel GL, Kuntz ID, Furth PS, Alvarez JC, Oritz De Montellano PR, Decamp DL, Babé LM and Craik CS, Structure-Based Design of Nonpeptide Inhibitors Specific for the Human Immunodeficiency Virus 1 Protease. *Proc Natl Acad Sci USA* 87: 6644-6648, 1990.

227. Kornbluth RS, Oh PS, Munis JR, Cleveland PH and Richman DD, The Role of Interferons in the Control of HIV Replication in Macrophages. *Clin Immunol Immunopathol 54:* 200-219, 1990.

228. Hansen JES, Nielsen CM, Nielsen C, Heegaard P, Mathiesen LR and Nielsen JO, Correlation Between Carbohydrate Structures on the Envelope Glycoprotein Gp120 of HIV-1 and HIV-2 and Syncytium Inhibition with Lectins. AIDS 3: 635-641, 1989.

229. Oh-Ishi T, Goldman CK, Misiti J and Waldmann TA, The interaction of Interleukin 2 with its receptor in the generation of suppressor T cells in antigen-specific and antigen-nonspecific systems in vitro. *Clin Immund Immunopathol 52:* 447-459, 1989.

230. Mizel SB, Kilian PL, Lewis JC, Paganelli KA and Chizzonite RA, The Interleukin 1 receptor. Dynamics of Interleukin 1 binding and internalization in T cells and fibroblasts. J *Immunol* 138: 2906-2912, 1987.

231. Falk W, Vonhogen I and Krammer PH, Activation of T-Cells by Interleukin-1 Involves Internalization of Interleukin-1. *Lymphokine Res* 8: 263-268, 1989.

232. Yagita H, Nakata M, Azuma A, Nitta T, Takeshita T, Sugamura K and Okumura K, Activation of peripheral blood T cells via the p75 Interleukin 2 receptor. J *Exp Med* 170: 1445-1450, 1989.

233. Peters DK and Norback DH, Binding and internalization of biotinylated Interleukin-2 in human lymphocytes. *Blood* 76: 97-104, 1990.

234. Duprez V and Dautry-Varsat A, Receptor-mediated endocytosis of interleukin 2 in a human tumor T cell line. J Bid *Chem* 261: 15450-15454, 1986.

235. Lee T-P and Mookerjee BK, Receptor-mediated endocytosis of IL2 by PHA stimulated

lymphocytes. *Transplant Proc* 21: 100-102,1989.
236. Zuber CE, Galizzi JP, Harada N, Durand I and Banchereau J, Interleukin-4 receptors on human blood mononuclear cells. *Cell Immunol* 129: 329-340,1990.
237. Wagteveld AJ, van Zanten AK, Esselink MT, Halie MR and Vellenga E, Expression and Regulation of IL-4 Receptors on Human Monocytes and Acute Myeloblastic Leukemic Cells. *Leukemia* 5: 782-788,1991.
238. Munck Petersen C, Davidsen O, Moestrup SK, Sonne O, Nykjaer A and Møller BK, Cellular targets and receptors for interleukin-6. II. Characterization of IL-6 binding and receptors in peripheral blood cells and macrophages. *Eur J Clin Invest* 20: 377-384, 1990.
239. Mancilla J, Schindler R, Dinarello CA, Importance of glycosylation for receptor biding and biological activity of IL-6. In: *Molecular and Cellular Biology of Cytokines* pp. 51-56. Wiley-Liss, 1990.
240. Moestrup SK, Kaltoft K, Munck Petersen C, Pedersen S, Gliemann J and Christensen El, Immunocytochemical identifiition of the human alpha-2-macroglobulin receptor in monocytes and fibroblasts: monoclonal antibodies define the receptor as a monocyte differentiation antigen. *Exp Cell Res 190:* 195-203, 1990.
241. De Jong G, Van Dijk JP and Van Eijk HG, The biology of transferrin. *Clin Chim Acta* 190: 1-46, 1990.
242. Pernis B, Internalization of lymphocyte membrane components. *Immunol Today* 6: 45-49, 1985.
243. Torres JM, Geuskens M and Uriel J, Receptor-Mediated Endocytosis and Recycling of Alpha-Fetoprotein in Human B-Lymphoma and T-Leukemia Cells. *Int J Cancer 47:* 110-117, 1991.
244. Pongor S, Ulrich PC, Benisath FA and Cerami A, Aging of proteins: isolation and identification of a fluorescent chromophore from the reactions of polypeptides with glucose. *Proc Natl Acad Sci USA* 81: *2684-2688,* 1984.
245. Vlassara H, Brownlee M and Cerami A, Novel macrophage receptor for glucose modified proteins is distinct from previously described scavenger receptors. J *Exp Med* 164: 1301-1309, 1986.
246. Vlassara H, Brownlee M and Cerami A, High affinity receptor mediated uptake and degradation of glucose modified proteins: a potential mechanism for the removal of senescent macromolecules. *Proc Natl Acad Sci USA* 82: 5588-5592, 1985.
247. Yang Z, Makita Z, Horii Y, Brunelle S, Cerami A, Sehajpal P, Suthanthiran M and Vlassara H, Two Novel Rat Liver Membrane Proteins That Bind Advanced Glycosylation Endproducts: Relationship to Macrophage Receptor for Glucose-Modified Proteins. J *Exp Med 174:* 515-524, 1991.
248. Tomaska LD and Parish CR, Inhibition of secondary IgG responses by N-acetyl-D-galactosamine. *Eur J Immunol* 11: 181-186, 1981.
249. Rom WN, Human Mononuclear Phagocytes Express the Insulin-Like Growth Factor-II/Mannose-6-Phosphate Receptor. *Am J Respir Cell M d Bid 4:* 555-559, 1991.
250. Johnson EW, Jones LA and Kozak RW, Expression and function of Insulin-like Growth Factor receptors on anti-CD3 activated human T lymphocytes. J *Immunol* 148: 63-71, 1992.
251. Mommaas AM, Wijsman MC, Verduijn W, Vermeer BJ and Claas FMJ, Internalization of MHC class I molecules is a prerequisite for endocytosis of endorphin by lymphocytes. *Clin Exp Immunol 84:* 170-174,1991.
252. Czop JK and Austen KF, A beta-glucan inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. J *Immunol* 134: 2588-2593, 1985.
253. Czop JK, Gurish MF and Kadish JL, Production and isolation of rabbit anti-idiotypic antibodies directed against the human monocyte receptor for yeast beta-glucans. J *Immunol* 145: 995-1001, 1990.
254. Lanzavecchia A, Roosnek E, Gregory T, Berman P and Abrignani S, T cells can present antigens such as HIV gp120 targeted to their own surface molecules. *Nature* 334: 530-532, 1988.

255. Monsigny M, Kieda C and Roche AC, Membrane lectins. *Bid Cell* 33: 289-300, 1979.
256. Weir DM, Surface carbohydrates and lectins in cellular recognition. *Immunol Today* august: 45-51, 1980.
257. Stoolman LM and Rosen SD, Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation. J *Cell Bid* 96: 722-729,1983.
258. Apgar JR and Cresswell P, Expression of cell surface lectins on activated human lymphoid cells. *Eur* J *Immunol* 12: 570-576,1982.
259. Teodorescu M and Mayer EP, Binding of bacteria to lymphocyte subpopulations. Adv *Immunol* 33: 307-351, 1982.
260. Teodorescu M, Binding of bacteria in lymphocyte subpopulations: role of lectin-carbohydrate interactions. *Biol Cell* 51: 251-258, 1984.
261. Seve A-P, Hubert J, Bouvier D, Bourgeois C, Midoux P, Roche AC and Monsigny M, Analysis of sugar-binding sites in mammalian cell nuclei by quantative flow microfluorometry. *Proc Natl Acad Sci USA 83:* 5997-6001, 1986.
262. Bourgeois CA, Seve AP, Monsigny M, Hubert J, Localization of lectins in the nuclear and the nucleolar structures of reptilian and mammalian cells. In: *Lectins-Biology, Biochemistry,Clinical Biochemistry* pp. 431-437. 1988.
263. Monsigny M, Hubert J, Obrénovitch A, Schrével J, Neoglycoproteins as tools to analyze endogenous lectins *and* glycoconjugates. In: *Electron Microscopy of Subcellular Dynamics* (Ed. Plattner H), pp. 239-263. CRC Press,Inc., Florida, 1989.
264. Decker JM, Lectin-like molecules on murine thymocytes and splenocytes-1. Molecules specific for the oligosaccharide moiety of the fetal alpha-globulin fetuin. *Mol Immunol* 17: 803-808, 1980.
265. Bezouska K, Karaskova H, Taborsky O, Kofronova O, Vorisek J, Kubrycht J, Kocourek J, Studies on lectins. LXVI. Binding of serum glycoprotein asialo-oligosaccharides to the porcine liver and leucocyte membrane lectins. In: *Lectins, Vd. IV* (Eds. Bog-Hansen TC and Breborowicz J), pp. 353-367. Walter de Gruyter & Co., Berlin, 1985.
266. Lee H, Kelm S, Yoshino T and Schauer R, Carbohydrate specificity of the galactose-recognizing receptor of rat peritoneal macrophages. *Bid Chem Hoppe Seyler 369:* 705-714, 1988.
267. Pimpaneau V, Midoux P, Durand G, Debaetselier P, Monsigny M and Roche AC, Endocytosis of Alpha-1-Acid Glycoprotein Variants and of Neoglycoproteins Containing Mannose Derivatives by a Mouse Hybridoma Cell Line (2Cll-12) - Comparison with Mouse Peritoneal Macrophages. *Glycocon* J 6: 561-574, 1989.
268. Tomoda H, Kishimoto Y and Lee YC, Temperature Effect on Endocytosis and Exocytosis by Rabbit Alveolar Macrophages. J *Bid Chem 264:* 15445-15450, 1989.
269. Roche AC, Barzilay M, Midoux P, Junqua S, Sharon N and Monsigny M, Sugar-specific endocytosis of glycoproteins by Lewis lung carcinoma cells. *J Cell Biochem 22:* 131-140, 1983.
270. Ralapati S, Lee CMJ, Teodorescu M, Membrane lectins of human lymphocytes. In: *15th International Leucocyte Culture Conference, Asilomar* , 1985.
271. Baum LG and Cresswell P, Isolation and Characterization of a Galactose-Specific Carbohydrate Binding Protein from Human Lymphoblastic Cells. *Biochem Biophys Res Commun* 173: 1079-1085, 1990.
272. Grillon C, Monsigny M and Kieda C, Soluble Human Lymphocyte Sugar Binding Proteins with Immunosuppressive Activity. *Immunol Lett* 28: 47-56, 1991.
273. Barzilay M, Monsigny M, Sharon N, Interaction of soybean agglutinin with human peripheral blood lymphocyte subpopulations: evidence for the existence of a lectin-like substance on the lymphocyte surface. In: *Lectins-Biology,Biochemistry,Clinical Biochemistry* pp. 67-81. De Gruyter, W., Berlin/NY, 1982.
274. Lennartz MR, Sessions Cole F and Stahl PD, Biosynthesis and processing of the mannose receptor in human macrophages. J *Bid Chem* 264: 2385-2390, 1989.
275. Roche AC, Midoux P, Bouchard P and Monsigny M, Membrane lectins on human monocytes. Maturation-dependent modulation of 6-phosphomannose and mannose receptors. *FEBS Lett* 193: 63–68, *1985.*

Pimpaneau V, Midoux P, **Monsigny M** and **Roche AC,** Characterization **and Isolation** of an **Intracellular** D-Mannose-Specific Receptor from **Human Promyelocytic** HL60 **Cells.** ***Carbohydr Res 2U:*** **95-108, 1991.**

277. **Grillon** C, **Monsigny M** and Kieda C, Cell surface lectins **of** human **granulocytes: their expression** is modulated by **mononuclear cells and granulocyte/macrophage** colony-stimulatingfactor. ***Glycobiology*** **1: 33-38, 1990.**
278. Kieda CMT, **Bowles** DJ, Ravid A and Sharon N, Lectins in lymphocyte membranes. ***FEBS Lett*** 94: **391-3%, 1978.**
279. **Kieda** C, **Roche** AC, **Delmotte** F and **Monsigny M,** Lymphocyte membrane **lectins.** Direct visualization by the use of **fluoresceinyl-glycosylated cytochemical** markers. ***FEBS Lett*** 99: **329-332, 1979.**
280. **Brock** J, Schulze H-A, Neels P, **Fischer A, Bremer** H and **Walzel** H, **Detection** of Mannose-Binding Proteins on Mouse Lymphocytes. ***Eur J Cell Bid 54:*** **90-94, 1991.**
281. Popovic **M and Gartner** S, Biology **of** human **immunodeficiency** virus: virus receptor ***and*** cell tropism. ***Curr*** Op ***Immunol*** **1: 516-520, 1989.**
282. **Chong** ASF and Parish CR, **Cell** surface receptors for **sulphated polysaccharides:** a potential marker for **macrophage** subsets. ***Immunology*** 58: **277-284, 1986.**
283. **Monsigny** M, **Roche** AC **and Midoux** P, Uptake of **neoglycoproteins** via membrane **lectin(s)** of **L1210 cells** evidenced by quantitative **cytofluorometry** and **drug targeting.** ***Bid Cell*** **51: 187-1%, 1984.**
284. **Frigeri LG** and **Liu PT,** Surface Expression of Functional IgE Binding Protein, an Endogenous Lectin, on Mast **Cells** and **Macrophages.** J ***Immunol*** **148: 861-867, 1992.**
285. **Shepherd VL, Lee** YC, **Schlesinger** PH and **Stahl** PD, **L-Fucose-terminated glycoconjugates are** recognized by **pinocytosis** receptors on macrophages. ***Proc Natl Acad Sci USA 78:*** **1019-1022, 1981.**

286a. **Ezekowitz** RAB, Williams DJ, **Koziel** H, **Armstrong MYK,** Warner A, **Richards FF and** Rose RM, Uptake of **Pneumocystis** Carinii Mediated by the **Macrophage** Mannose Receptor. ***Nature*** **351: 155-158, 1991.**

286b. **Van Berkel** TJC, **Kruyt** JK, **Harkes** L, **Nagelkerke JF, Spanjer** H and **Kempen HJM,** Receptor-dependent **targeting** of native and modified lipoproteins to liver **cells. In:** ***Site-Specific Drug Delivery*** **(Eds. Tomlinson** E and Davis SS), John **Wiley** and Sons Ltd, **pp.49-68, 1986.**

287. **Stahl** PD, **Somsel Rodman J,** Miller MJ and **Schlesinger PH,** Evidence for receptor-mediated binding of **glycoproteins, glycoconjugates** and **lysosomal glycosidases** by alveolar macrophages. ***Proc Natl Acad Sci USA 75:*** **1399-1403, 1978.**
288. **Boldt** DH and **Armstrong** JP, Rosette formation between human lymphocytes and sheep **erythrocytes.** Inhibition of rosette formation by specific **glycopeptides.** J ***Clin Invest 57:*** **1068-1078, 1976.**
289. Rosen SD, Geoffrey JS, Lasky LA, S i r MS, **Stachel** S, **Stoolman** LM, True DD, **Yednock TA,** A lymphocyte homing receptor is a **lectin,** a member of the emerging LEC-CAM family. In: ***Glycobiology*** **(Eds.** Welply **JK** and **Jaworski** E), pp. **69-90. Wiley-Liss,Inc.,** New York, **1990.**
290. Berg EL, **Goldstein** LA, Picker LJ, **Streeter** PR, **Jutila** MA, Bargatze RF, **Zhou DFH,** Butcher EC, Lymphocyte homing receptors and vascular **adressins.** In: ***Glycobidogy*** **(Eds.** Welply JK and **Jaworski** E), pp. **91-105. Wiley-Liss,Inc.,** New **York, 1990.**
291. Butcher EC, The role of carbohydrate in **heterotypic cell-cell** recognition: lymphocyte-high **endothelial** cell interaction as a model system. ***Biol Cell*** 51: **247-250, 1984.**
292. **Brandley** BK, **Swiedler** SJ and **Robbins** PW, Carbohydrate Ligands of the LEC **Cell Adhesion Molecules.** ***Cell*** 63: **861-863. 1990.**
293. Jutila **MA, Kishimoto** TK and Butcher EC, Regulation and **lectin** activity of the human **neutrophil** peripheral lymph node homing receptor. ***Blood*** 76: **178-183, 1990.**
294. **Stoolman LM,** Adhesion **molecules** controlling lymphocyte migration. ***Cell*** 56: **907-910, 1989.**
295. **Ezekowitz** RAB, Sastry K, **Bailly** P and Warner **A,** Molecular Characterization of the **Human Macrophage Mannose** Receptor: Demonstration of Multiple Carbohydrate

Recognition-Like Domains and Phagocytosis of Yeasts in Cos-1 Cells. J *Exp Med* 172: 1785-1794, 1990.

2%. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Hirsch MS, Jackson GG, Durack DT and Nusinoff-Lehrman S, The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl* J *Med* 317: 192-197,1987.

297. Richman DD, Grimes JM and Lagakos SW, Effect of Stage of Disease and Drug Dose on Zidovudine Susceptibilities of Isolates of Human Immunodeficiency Virus. J *Acquir Immune Defic Syndr* 3: 743-746,1990.

298. Laskin OL, De Miranda P and Blum MR, Azidothymidine steady-state pharmacokinetics in patients with AIDS and AIDS-dated complex. *J Infect Dis* 159: 745-747,1989.

299. Stevenson M, Stanwick TL, Dempsey MP and Lamonica CA, HIV-1 Replication Is Controlled at the Level of T Cell Activation and Proviral Integration. *EMBO* J 9: 1551-1560, 1990.